Proceedings

Volume 68, 2017

American Society of Animal Science
Western Section

Fargo, North Dakota
June 20–23, 2017

ASAS Western Section

FUTURE MEETING LOCATIONS

2018	**Bend, Oregon**
2019	**Boise, Idaho**
2020	**Sacramento, California**

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MEETING SPONSORS

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North Dakota State University Department of Animal Sciences
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Zinpro Corporation

First Place Recipient of the Applied Animal Science Awards

Western Section, American Society of Animal Science

Undergraduate Poster Competition

Beaver Creek Farm

Graduate Student Paper Competition

Western Section, American Society of Animal Science

2016-17 WESTERN SECTION COMMITTEES

EXECUTIVE

S. Ivey, President - New Mexico State University
C. Larson, President-Elect - Zinpro Corporation, Eden Prairie, MN
M. Salisbury, Past President - San Angelo University
K. Vonnahme, Secretary-Treasurer - North Dakota State University
R. Cooke, A & C Chair - Oregon State University
G. Duff, ASAS Director - New Mexico State University
B. Carter, Industry Director - PerforMix
S. Prosser, Graduate Student Representative - New Mexico State University
H. Cunningham, Graduate Student Representative - University of Wyoming

AWARD (3-YEAR TERM)

‡*M. Salisbury - San Angelo State University (2016-17)
R. Funston - University of Nebraska (2014-17)
G. Moss - University of Wyoming (2014-17)
C. Loest - New Mexico State University (2015 - 18)
S. Archibeque - Colorado State University (2015-18)
M. Hubbert - New Mexico State University (2016-19)
D. Tolleson - Texas Agrilife, Sonora TX (2016-19)

BEEF SYMPOSIUM (3 YEAR TERM)

*E. Schollegerdes - New Mexico State University (2015-18)
M. Ward - New Mexico State University (2014-17)
D. Bohnert - Oregon State University (2015-18)
C. Runyan - Angelo State University (2015-18)
R. Waterman - MT-USDA (2015-18)
M. Van Emon - Montana State University (2016-19)
C. Dahlen - North Dakota State University (2016-19)

ADVISING AND COORDINATING (3 YEAR TERM)

*R. Cooke - Oregon State University (2016-17)
‡C. Larson - Zinpro Corporation (2016-17)
‡H. Cunningham, University of Wyoming (2016-17)
H. Neiburgs, Washington State University (2014-17)
K. Cammack, University of Wyoming (2014-17)
S. Soto - New Mexico State University (2015-18)
L. Prezotto - MT Research Station (2015-18)
B. Glaze - University of Idaho (2016-19)

PAPER COMPETITION (2 YEAR TERM)

*C. Schauer, North Dakota State University (2015-16)
A. Summers - New Mexico State University (2015-17)
J. Gifford - Oklahoma State University (2015 - 17)
W. Stewart - Montana State University (2015-17)
M. Ellison - University of Idaho (2015-18)
T. Mulliniks - University of Tennessee (2016-18)
M. Beckman - Zinpro Corporation (2016-18)
J. Kincheloe - North Dakota State University (2016-18)
D. Yates - University of Nebraska-Lincoln (2016-18)

ACADEMIC QUADRATHLON

*R. Endecott - Montana State University
M. Kennedy - Oregon State University
D. Faulkner - University of Arizona
A. Stalker - BYU-Idaho
K. DeAtley - Chico State University
S. Archibeque - Colorado State University
H. Han - Colorado State University
S. Soto - New Mexico State University
B. Bowman, - Utah State University
D. Rule - University of Wyoming

NECROLOGY

‡*M. Salisbury -San Angelo State University

NOMINATING

‡**M. Salisbury - San Angelo State University
‡J. Berardinelli - Montana State University
‡J. Brett Taylor - USDA, ARS - Dubois, ID

ASAS WESTERN SECTION YOUNG SCHOLARS PROGRAM

*R. Ashley ,New Mexico State University (2015-17)
B. Alexander, University of Wyoming (2015-17)
R. Cook, Oregon State University (2015-17)
‡H. Cunningham, Graduate Student Representative, University of Wyoming (2016-17)
C. Loest - New Mexico State University (2016-19)
R. Endecott - Montana State University (2016-19)
K. Dorton - Central Life Sciences (2016-19)

UNDERGRADUATE POSTER COMPETITION (2 YEAR TERM)

*K. DeAtley - Chico State University (2016-18)
T. Geary - USDA ARS, Ft. Keogh, Miles City, MT (2015-17)
S. Prosser - New Mexico State University (2016-18)

STRATEGIC PLANNING COMMITTEE

*C. Larson, Zinpro Corporation, Eden Prairie, MN
J. Sprinkle, University of Arizona
R. Cooke, Oregon State University
K. DeAtley, Chico State University
A. Roberts, USDA/ARS Fort Keogh
S. Ivey, New Mexico State University
E. Sherman, Industry, private research facility

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* Chair
‡ Mandatory, not appointed
§ Not appointed by WSASAS President

2017 WSASAS ANNUAL BUSINESS MEETING

Salt Lake City, Utah

Prepared by: Kimberly Vonnahme, *A&C Chair*

CALL TO ORDER: The meeting was called to order at 7:45 am, Friday, July 22.

APPROVAL OF THE AGENDA: A motion to approve the agenda was put forth by John Hall, Seconded by Ken Olson. Motion Passed.

APPROVAL OF THE MINUTES: Minutes were considered and a motion to approve the minutes was put forth by Ken Olson, seconded by Rachel Endecott. No discussion. Motion passes.

Reports

FINANCIAL REPORT: Jacelyn Hemmelgarn reported that the decrease we see in net assets is reflective of the fact that investments are down. David Bohnert commented on the decreasing trend of net assets over the last 5 years. It was commented that the investments play a big role in that number. Ken Olson commented that we may want to consider raising proceedings costs. Olson further commented on raising registration and Beef Symposium registration rates. We need to adjust appropriately over time for these events. Boone Carter inquired what our investments are; it was relayed back that 4% of our investments come from ASAS. See Appendix 1.

2016 ANNUAL WSASAS MEETING: Mike Salisbury reported that WSASAS had 205 registered; 139 registered for the Beef Symposium; there were 142 that attended the Awards banquet; and there were 52 proceedings papers.

ASAS PRESIDENT: Dr. Mike Looper, President of ASAS, reported that the attendance for JAM, WSASAS and ISAG was ~4000.

Highlights include:

- ASAS has ~6000 in membership. There is increasing membership with a greater growth in the international membership. This reflects the mission to grow in diversity.
- Journal of Animal Science has an average submit to publication rate of 160 days
- The Impact factor was 2.0 with greater than 26K citations.
- Universities should inquire about having Editor-in-Chief, Jim Sartin, come out and provide information on submission requirements to JAS.
- Bylaw changes are being discussed at the ASAS Business meeting and he encouraged our presence at that meeting.
- ASAS Strategic Plan needs to be updated.
- Innovate meetings in September on Animal Health
- Upcoming meetings include: 2017-Baltimore; 2018-Vancouver

WSASAS PRESIDENT: Mike Salisbury gave the president's report. The strategic plan for WSASAS needs to be worked through. Kim Vonnahme was voted in as Secretary/Treasurer. Reinaldo Cooke will be the new A&C chair and the 2018 meeting will be head in Oregon. There is a continued need to smooth the submission process of the proceedings papers. Shanna Ivey discussed that we can submit the proceedings papers via Manuscript Central. This will allow the proceedings to be searchable and edited by the technical editor. For the graduate competition, the original submission will be provided to the judges for the competition piece. There will be no extra cost to use manuscript central and the submission deadline will be similar. Dr. Salisbury also commented that we need to plan ahead 3 years for our meetings to allow enough planning time.

WSASAS Committees

ACADEMIC QUADRATHLON: Rachel Endecott reported that the 2016 Western Section AQ was hosted by Chico State on April 15-16 with 4 teams participating. Results as follows:

1. Chico State
2. Montana State (tie)
2. New Mexico State (tie)
4. Oregon State

Next year, the competition will be held at OSU. Matt Kennedy will become the chair.

ADVISING AND COORDINATING: Kim Vonnahme reported the A&C Committee discussions. A full report is in Appendix 2.

AWARDS: Jim Berardinelli thanked the members of the awards committee as all reviewers reviewed and had timely ranking. March 28th was submission date, we need to make sure the guidelines are on the website. See Appendix 3.

GLNC: Ken Olson. The GLNC started on Sunday. There were 16 oral presentations and there were invited posters as well. There were 21 volunteer posters. There were 139 registrants. The proceedings from this event will be posted on the JAS website. Funding from this event came from the annual beef symposium budget as well as support from the National office. The support was greatly appreciated.

GRADUATE STUDENT PAPER COMPETITION: Jim Oltjen reported that there were 17 papers with 9 institutions represented with 5 being eligible for the institution award. Just a reminder that students must be WSASAS members. Recommendations from the judges. There must be an IACUC statement. For the institutional award, it was suggested that the top 2 scores from each institution be used. The other option is to use the average of the scores. It was discussed by several members that the objective of the institution award is should be about the institution, not necessarily the individual talks. There was discussion about creating an index. Other commented that the average may be a better indicator, and would keep it less complicated. It was suggested to have the A&C committee and Graduate Competition committee work together to come up with a plan. See Appendix 4.

GRADUATE STUDENT REPRESENTATIVE: Hannah Cunningham reported that there were 68 at the graduate student Lunch and Learn. Evaluations were done to decide on next year's topic. The mixer had good attendance. Stacia Prosser is the new representative. Thank you to Kelcey Quinn for her service.

NECROLOGY: Jim Berardinelli reported that Dr. James Oldfield passed. A moment of silence was given. See Appendix 5.

NOMINATING: Jim Berardinelli reported that the WSASAS representative to ASAS is Glenn Duff. Please nominate your colleagues for leadership roles in our society. See Appendix 6.

UNDERGRADUATE STUDENT POSTER COMPETITION: Kasey DeAtley reported that the time was a little tight to judge. If we continue to use ePosters, there will need to be some adjustments to the guidelines. As of now, there are only abstracts that are submitted (i.e. no undergraduate proceedings papers). Would like to determine if there could be an option to submit a proceedings paper if someone is interested. See Appendix 7.

YOUNG SCHOLARS PROGRAM: Brenda Alexander reported that the PhD recipient for the WSASAS Young Scholar was Rodrigo da Silva Marques from Oregon State University. There were 2 MS recipients: Amy Abrams from University of Wyoming and Stacia Prosser, New Mexico State University. Boone Carter made the suggestion that perhaps the Young Scholars could give a perspective at the awards banquet.

No other Old Business was discussed.

New Business

Proceedings on CDs for this meeting will be placed in the mail.

Future site selection discussion included Idaho for 2019. Idaho agreed. It was moved by Berardinelli and seconded by Endecott. Motion passes. There is a new site selection form that needs to be completed.

For the 2020 meeting, discussion to have it joint with Sacramento resulted in the following Pros: It exposes the grad students to a bigger meeting and the ASAS; it helps drawn in others to the GLNC; it is cheaper to attend the joint meetings if a person wishes to attend WSASAS and ASAS; The Cons: Loss of the WSASAS "feel". It was moved that WSASAS be held in 2020 in Sacramento by Joel Caton; Seconded by Jim Oltjen. Motion passes.

Kim Vonnahme welcomed everyone to attend the 2017 meeting in Fargo.

It was requested that the A&C and Exec committees give consideration to newer faculty to serve on the WSASAS committees. Moreover, it is important to keep the membership informed of which committees members are part of. Shanna Ivey stated that there are 6 open committee assignments and that she will be slating new people to committees. Any suggestions should be sent to her as she fills these

positions. IT is important that there is an industry representative on the Applied Animal Science committee.

It was suggested that we have a Dr. Hallford Appreciation Club with the revenue going towards graduate student travel to the WSASAS meetings. If Dr. Hallford accepts this, it was moved that $25K be raised with 4% going towards graduate student travel yearly. It was moved by John Hall and seconded by Sergio Soto-Navarro. Motion passed.

APPENDIX 1

Statement of Activities

	YTD 12/31/16
Revenue and Support	
Registrations	$4,120
Sponsorships	4,465
Proceedings	2,660
Investment Earnings Gain (Loss)	1,269
Total Revenue and Support	12,514
Expenses	
Printing	3,500
Awards/Plaques	8,982
Events	—
Marketing	—
Proceedings	2,899
Postage, Shipping & Supplies	1,034
Miscellaneous	1,479
Staff Support	—
Total Expenses	17,894
Change in Net Assets	(5,380)
Net Assets, Beginning of Period	42,892
Net Assets, End of Period	$37,512

APPENDIX 2

Advising and Coordinating Committee Report
July 20, 2016

MEMBERS PRESENT: Shanna Ivey, Kristi Cammack, Sergio Soto-Navarro, Connie Larson, Ligia Prezotto, Reinaldo Cooke, Kimberly Vonnahme

Members of the committee met at JAM and discussed issues dealing with symposia rotation, teaching webinars, graduate students competition, and meeting site selection.

The A&C committee recommends that the beef symposium be held on an annual basis. The committee also recommends that the host institution has the option to do another symposium at their discretion.

The A&C committee recommends that a 3 person committee be formed to try a teaching webinar series. This would greatly benefit members of the WSASAS that have greater teaching appointments. Some suggestions include: having the webinar be free to WSASAS members, but available to national ASAS members, having the webinar during fall and spring semester, and be short in duration (~1 hour).

FOR GRADUATE COMPETITION PAPERS: The A&C committee acknowledges the importance of animal approval by local institutions to perform animal research, and it is important that our students understand this importance. The A&C recommends that the guidelines for competing students and judges include a statement to assure this is clearly worded. We recommend having one of the 2 statements included in the proceeding and presentation: 1) This project was approved by the animal care and use committee of [insert the institution]; 2) This project did not require IACUC approval.

The A&C committee recommends that a judge can not judge a presentation from their institution. We request that the titles, authors, and institutions of the presentations that were submitted for competition be available to the Graduate Competition chair within 1 month to ensure that there are adequate numbers of reviewers for each paper. The chair can seek ad hoc reviewers to serve as judges if needed.

The A&C committee recommends that the Graduate Competition committee include a representative from institutions that have participated over the last 3 years. The President Elect has the authority to remove and replace a person who has not participated in committee responsibilities within a given year.

FOR THE INSTITUTION AWARD OF THE GRADUATE COMPETITION: The A&C committee recommends that the judges average the scores of all presenters within an eligible institution for the institution award.

THE YOUNG SCHOLAR AWARD: The A&C committee recommends that the Young Scholar application deadline be moved up and the selection of the award be made a minimum of 30 days prior to the proceedings submission. We further recommend that a student selected for the Young Scholar is still eligible to compete in the competition.

MEETING SITE SELECTION: The A&C committee recommends that the form for potential meeting sites be posted online immediately so that future selection sites may be made a minimum of 2 years in advance.

APPENDIX 3

July 18, 2016

MEMBERS PRESENT: July 18, 2016

TO: Members of the Western Section, American Society of Animal Science

2016 WSASAS Awards Committee Report

Prepared by James G. Berardinelli, Past-President

COMMITTEE MEMBERS:
James Berardinelli, Montana State University (Chair)
Scott Lake, University of Wyoming
Kimberly Vonnahme, North Dakota State University
Rick Funston, University of Nebraska
Gary Moss, University of Wyoming
Clint Loest, New Mexico State University
Shawn Archibeque - Colorado State University

Call for WSASAS award nominations was sent via e-mail to the membership and posted on the WSASAS 2016 Annual Meeting site on March 8, 2016. The deadline for nominations was March 28, 2016. Nominations were submitted through the WSASAS web site electronically using "WizeHive". "2016 Western Section Award Guidelines" were also posted on the WSASAS Annual Meeting web site.

Nominees by Award were:

Distinguished Service Award (established 1971)
- Dr. Connie Clark, Zinpro Corporation
- Dr. Michael Galyean, Texas Tech University
- Dr. N. Andy Cole, USDA, ARS, Texas

Distinguished Teacher Award (established, 1987)
- Dr. Ryan Ashley, New Mexico State University
- Dr. Shawn Archibeque, Colorado State University

Extension Award (established, 1987)
- Dr. Doug R. Tolleson, University of Arizona; currently, Texas A & M University

Young Scientist Award (established, 1975)
- Dr. Craig Gifford, Oklahoma State University; currently, New Mexico State University
- Dr. Jennifer Martin, Colorado State University
- Dr. Kasey DeAtley, California State University, Chico
- Dr. Reinaldo Cooke, Oregon State University, EO-ARC, Burns

The charge to the Committee members for review was to "Review the guidelines for the ASAS Western Section Awards to ensure you are judging the award based on the appropriate criteria" for each award. Each member was then asked to evaluate each nominee for each award on a scale of 1 to 5 (1 = most qualified; 5 = least qualified); unless there was a "conflict-of-interest" (COI) between a Committee member and a nominee. Dr. Shawn Archibeque declared a COI for review of the Distinguished Teacher Award because he was a nominee. One nominee, for the Distinguished Service Award (Dr. Connie Clark), was not reviewed due to the eligibility rule for WSASAS Awards which states: "Eligibility for Nomination: 1) A nominee must be a member of the Western Section of the American Society of Animal Science, but may not be an officer of the regional organization at the time of nomination." Dr. Larson is a current member (Secretary-Treasurer) of the WSASAS Executive Committee.

Ratings of each nominee for each award were then submitted by each Committee member to ASAS office via WizeHive. Mrs. Jacelyn Hemmelgarn, Chief Operations Officer of the ASAS, reported the ratings and comments of the Committee's evaluations of nominees for each award to the Chair, Jim Berardinelli. The Chair and Mrs. Hemmelgarn verified the top ratings and the award winner's qualifications. The Chair sent the names and salutations of each winner by e-mail to WSASAS Executive Committee for approval. The Chair contacted the nominators of each award winner by e-mail so that they could notified their respective award winner, and requested that each be present at the Annual Meeting to introduce and present their respective award winner with a plaque and monetary award. The Chair then sent, by e-mail, a personalized (signed) "letter of notification of award" to each award winner, highlighting the impressive comments by Committee Members and meritorious qualifications of each winner. Additionally, the Chair sent, by e-mail, a message to each of the nominators of non-award winners expressing the WSASAS thanks for their time, effort, and eloquent

nominations, time, efforts in this important matter of business of the Western Section, and requested that they consider nominating that individual for next year's award cycle. Lastly, the Chair recognized and expressed appreciation to the members of the 2016 WSASAS Awards Committee for their diligence, time, effort, and expertise in the review of the nominees and selections of the award winners.

The 2016 Award winners and Nominators for each award were:

THE DISTINGUISHED SERVICE AWARD

Dr. Michael Galyean, Texas Tech University
Nominator—Dr. Mark Branine (Zinpro Corp.) and Dr. Micheal Hubbard (NMSU)

THE DISTINGUISHED TEACHER AWARD

Dr. Ryan Ashley, New Mexico State University
Nominator—Ms. Stacia Z. Prosser, Graduate Student, New Mexico State University

THE EXTENSION AWARD

Dr. Doug Tolleson, University of Arizona; currently, Texas A & M University
Nominator—Dr. James E. Sprinkle, University of Idaho

THE YOUNG SCIENTIST AWARD

Dr. Reinaldo Cooke, Oregon State University, EOARC, Burns
Nominator—Dr. David Bohnert, Oregon State University, EOARC, Burns

PRESENTED TO THE MEMBERSHIP

WSASAS Annual Business Meeting
Salt Lake City, UT
22 July 2016 (amended and submitted to the Secretary-Treasurer, 28 July, 2016)

James G. Berardinelli, Chair

APPENDIX 4

Graduate Student Paper Competition Final Report
June 20, 2016

COMMITTEE MEMBERS: Christopher Schauer (NDSU; Chair), Lauren Hanna (NDSU), James Oltjen (UC, Davis), Dan Faulkner (UA), Tanja Hess (CSU), A. Summers (NMSU), Jennifer Gifford (NMSU), Whit Stewart (MSU), Melanie Beckman (Zinpro), and S. Trojan (TTU).

Five committee members were in attendance at the 2016 meeting including: C. Schauer, J. Oltjen, A. Summers, J. Gifford, and M. Beckman. Additional written scores were submitted by L Hanna and W. Stewart. S. Trojan, D. Faulkner, and T. Hess did not participate in any of committee's tasks.

Seventeen proceedings were submitted for competition from nine institutions and all were accepted for presentation. Prior to acceptance, abstracts were evaluated for evidence of hypothesis driven research and statistical analysis. Proceedings were accepted as submitted with no request for revised manuscripts/ abstracts.

Proceedings and presentations were all high-quality. For scoring, an average score of the written (50 possible points) and oral presentation (50 possible points) was calculated and then combined for a total score (100 points). The top five placing were: 1). E.R. Oosthuysen, 2). M.S. Crouse, 3) C.S. Hebbert, 4) J.J. Kincheloe, and 5) K.M. Schubach.

Institutions were deemed eligible for the institutional award with two competitors. Institutions eligible for the 2016 award were: New Mexico State University (n=3), North Dakota State University (n=3), Oregon State University (n=3), University of Wyoming (2), and the University of Nebraska (2). Colorado State University, Montana State University, UC Davis, and South Dakota State University were not eligible for the institutional award with only one competitor from each institution. For scoring, the top two scores from each institution were used to calculate a total institution score. Using this matrix New Mexico State University was the top scoring institution with students placing 1st and 3rd, with a combined score of 185.3. North Dakota State University placed 2nd, with students placing 2nd and 6th and a combined score of 181.7.

Future consideration needs be given to allowing the Chair to find proxies or replacements for committee members that cannot attend. We added a 10th judge 5 days prior to the meeting as we were down to 4 judges planning on being in attendance for the oral competition.

Per the committee's discussions last year and the WSASAS Annual Meeting minutes, the committee conducted a conference call on 6/15/2016 and has provided the following recommendations to be considered by the board for adoption as part of the rules of the WSASAS Graduate Student Competition:

Revisions of guidelines approved to be forwarded to board

INSTITUTIONAL AWARD: To be eligible for the Institutional Award, a minimum of 2 students participating is required. The highest 2 scores from each institution will be used to calculate the Institutional Award winner.

CONFLICT OF INTEREST FOR GRADING: Committee members cannot grade any paper/presentation for their own students or if they are listed as an author on a paper. However, they can grade students within their own institution, as long as the committee member is not listed as an author.

IACUC Statement suggested wording to be added to the guidelines for authors: "If the research project required IACUC approval, please include the Approval # in the Materials and Methods.

Sincerely,

Christopher Schauer, Chair
North Dakota State University

APPENDIX 5

July 18, 2016

TO: Members of the Western Section, American Society of Animal Science

2016 WSASAS Necrology Report

Prepared by James G. Berardinelli, *Past-President*

WSASAS membership was reminded, via e-mail from Ms. Jacelyn Hemmelgarn of ASAS, to notify Jim Berardinelli of the passing of past and present WSASAS members, and affiliated members of the Section during the past year (2105-2016) May 18, 2016.

Jim Berardinelli received 1 name submitted by Dr. David Bohnert on April 6, 2016, originally sent to by Dr. Killefer, Department Head, Oregon State University. Dr. Killefer wrote,

> *"I am passing along the sad news of Dr. James Oldfield's passing. Dr. Oldfield has been so very instrumental in not only helping shape the culture of Animal Sciences at OSU and across the state and nation, but he has had a tremendous influence on the personal lives of many of us. We will miss Jim as he truly embodied what it is to be a "Gentleman and Scholar".*

Dr. Oldfield was 94 years of age when he passed away on Sunday, April 3, 2016.

Dr. Oldfield obituary can be viewed: http://www.legacy.com/obituaries/statesmanjournal/obituary.aspx?pid=179608326

PRESENTED TO THE MEMBERSHIP
WSASAS Annual Business Meeting
Salt Lake City, UT
22 July 2016 (amended and submitted to the Secretary-Treasurer, 28 July, 2016)

James G. Berardinelli, Chair

APPENDIX 6

July 18, 2016

TO: Members of the Western Section, American Society of Animal Science

2016 WSASAS Nominating Committee Report

Prepared by James G. Berardinelli, Past-President

COMMITTEE MEMBERS:
Glenn Duff, New Mexico State University
J. Bret Taylor, USDA, ARS, Dubois, ID

For the 2016-2017 election cycle, the Western Section had the following three position on the WSASAS Executive Committee: 1) Secretary-Treasurer; 2) WSASAS Representative to the National Board; and, 3) Western Section Graduate Director. The "Notice of Election" was e-mail to the membership by Ms. Jacelyn Hemmelgarn May 18, 2016, and posted on the WSASAS 2016 Meeting web site for electronic voting, along with biographical sketches for each nominee. Notice was given that, "If you have any questions about the election site, contact Jacelyn Hemmelgarn (jacelynh@asas.org, 217-689-2432) or Melissa Burnett (melissab@asas.org, 217-689-2433) to receive a paper copy of the candidates' biographical information.

The voting deadline was posted as 11:59pm CDT on June 24, 2016. The Election actually closed on June 28, 2016.

Nominees were:

FOR SECRETARY-TREASURER

Dr. Kimberly Vonnahme, North Dakota State University (unopposed)

FOR NATIONAL ASAS DIRECTOR, WESTERN SECTION (listed on ballot as 'Western Section Representative to the National Board')

Dr. Glenn Duff, New Mexico State University
Dr. Scott Lake, University of Wyoming

WESTERN SECTION GRADUATE DIRECTOR

Noe Alberto Gomez, University of California, Davis
Stacia Prosser, New Mexico State University
Shelby Rosasco, New Mexico State University

Results of the Election were:

FOR SECRETARY-TREASURER (1-yr term; 2016-2017)
Dr. Kimberly Vonnahme, North Dakota State University

FOR NATIONAL ASAS DIRECTOR, WESTERN SECTION (3-yr term; 2016-2019)
Dr. Glenn Duff, New Mexico State University

FOR WESTERN SECTION GRADUATE DIRECTOR (2-yr term; 2016-2018)*
Ms. Stacia Prosser, New Mexico State University

*****Mr. Noe Alberto Gomez, University of California, Davis, won the election by 1 vote. However, the Chair was notified by Mr. Gomez on July, 13, 2016, that he would be unable to perform the duties of this office due to personal reasons. After Executive Committee approval, Ms. Prosser was notified by Ms. Kelsey Quinn (out-going Graduate Representative) that she was the next highest vote-getter and elected as the in-coming WSASAS Graduate Representative. The other were 2 other candidates for the WSASAS Graduate Director Position were notified by the Chair via e-mail of the results of the election and were encouraged to submit their qualifications for this position in the future.

Lastly, the Chair recognized and expressed appreciation to the members of the 2016 WSASAS Nominating Committee and Mrs. Jacelyn Hemmelgarn, Chief Operations Officer of the ASAS, for their diligence, time, effort, and expertise in the review of the nominees for these positions and management of the process for the 2016 WSASAS election.

PRESENTED TO THE MEMBERSHIP
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James G. Berardinelli, Chair

APPENDIX 7

Undergraduate Poster Competition Committee Report

The third annual undergraduate poster competition was conducted on Wednesday, July 20, 2016 at the Salt Lake City Convention Center (Salt Lake City, UT). A total of six undergraduates submitted abstracts for the poster session. Institutions represented included California State University, Chico, North Dakota State Uniersity, and North Carolina State University. Committee members that served as judges included Dr. Rachel Endecott, Dr. Kristi Cammack, Dr. Scott Spiedel, and Dr. Boone Carter.

Winners of the poster session were:

- 1st Place: G.E. Woodmansee, California State University, Chico
- 2nd Place: S.E. Butterfield, California State University, Chico
- 3rd Place: L. Huffacker, California State University, Chico

Monetary awards included $250 for 1st Place, $150 for 2nd Place, and $100 for 3rd place. Committee members are seeking additional sponsors for the 2017 contest and will be editing poster score sheet and procedures.

Respectfully submitted,
Kasey DeAtley, Chair

Interactions between dietary protein concentration, protein degradability, and beta-adrenergic agonist administration in finishing cattle[1]

K. L. Samuelson*[2,3], M. E. Hubbert†, and C. A. Löest*

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ABSTRACT: The objectives were to 1) summarize practices of consulting feedlot nutritionists, 2) evaluate effects of zilpaterol hydrocholoride (ZH) on performance and carcass characteristics of steers fed diets with increasing dietary RDP supplied as urea, and 3) determine if excess protein, supplied either as high RDP or high RUP, decreases performance of finishing cattle fed diets with or without ractopamine hydrochloride (RH). Consulting feedlot nutritionists (n = 24) completed a 101-question survey on receiving and finishing cattle management. The surveyed nutritionists indicated that grain byproducts were a prevalent feedstuff used by their clients (97.1%) in finishing diets. Approximately 84% of the nutritionists' clients used β-adrenergic agonists (βAA). The survey results indicate that cattle are likely receiving excess protein and a βAA in finishing diets. Cattle receiving a βAA may retain greater N and have decreased urea recycling. Therefore, we hypothesized that an interaction between βAA administration and dietary requirements for protein concentration and rumen degradability may exist. To test this hypothesis, 2 studies were conducted where finishing cattle were provided with the βAA, ZH or RH, for the last 27 or 35 d of the finishing period in combination with increasing concentrations of dietary protein supplied primarily as RDP or RUP. In the first experiment, no ZH × dietary RDP interactions ($P \geq 0.14$) occurred for all performance and carcass response variables. Feeding ZH for the last 27 d (included a 3-d withdrawal period) of the finishing period increased ($P < 0.01$) ADG, decreased ($P < 0.01$) DMI, and increased ($P < 0.01$) G:F compared with no ZH. Increasing dietary RDP linearly decreased ($P = 0.01$) ADG and DMI. In the second experiment, no RH × CP interactions ($P \geq 0.11$) occurred for performance or carcass traits. Excess CP did not affect ($P \geq 0.12$) final BW or ADG. Carcass-adjusted final BW and ADG tended to be greater ($P = 0.06$) for cattle receiving high RDP than high RUP and CON. Water intake, DMI, and G:F were not different ($P \geq 0.12$) among CP treatments. Water and DMI were not different ($P \geq 0.36$) for RH vs. no RH. Cattle receiving RH had greater ($P < 0.01$) final BW and ADG, and lower ($P < 0.01$) G:F compared with no RH. These results suggest that providing a βAA to finishing cattle does not increase their requirements for CP beyond that provided in a typical feedlot diet or influence the need for protein sources of varying degradability.

Key words: beta-agonist**,** cattle, protein
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INTRODUCTION

Repartitioning agents such as β-adrenergic agonists (**βAA**) have been used in the livestock industry to increase animal performance and alter composition of gain (Beermann, 2002). These compounds are used to affect target cells found within muscle or adipose tissue via downstream cellular signaling. In finishing cattle diets, provision of orally active βAA such as zilpaterol hydrochloride (**ZH**) and ractopamine hydrochloride (**RH**) results in improved ADG, HCW, and dressing percentage while decreasing carcass fat

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(Avendaño-Reyes et al., 2006; Vasconcelos et al., 2008; Arp et al., 2014). Previous research (Byrem et al., 1998; Brake et al., 2011; Parr et al., 2014) indicates that administration of βAA decreases N retention, serum urea N concentrations, and urea recycling while increasing AA uptake. This suggests that the improvements in performance associated with βAA administration are a result of greater N utilization by cattle receiving a repartitioning agent. Alternatively, it is possible that administration of βAA shifts the growth curve so that animals receiving a repartitioning agent have a greater capacity for growth than those receiving a traditional finishing diet with no βAA (Boyd et al., 1991). Regardless of the mechanism of action, because orally active βAA's increase muscle deposition, it is possible that they influence protein metabolism in a way that increases livestock nutrient requirements for dietary CP. Because net protein for muscle synthesis can come from both RDP and RUP, it is also possible that protein degradability may be important for cattle receiving a βAA. Therefore, we hypothesized that administration of a βAA would influence cattle requirements for dietary protein. The objectives of this research were to determine current management practices used in the finishing cattle industry and use these as parameters to investigate the interaction between dietary protein concentration, protein degradability, and βAA administration in finishing cattle.

MATERIALS AND METHODS

All procedures involving the use of human subjects were approved by the New Mexico State University Institutional Review Board, and all procedures involving the use of livestock were reviewed and approved by the New Mexico State Institutional Animal Care and Use Committee. The research methods used were completed as described by Samuelson et al. (2016a), Samuelson et al. (2016b), and Samuelson et al. (2016c).

RESULTS AND DISCUSSION

Awareness of the nutritional and management practices used for feedlot cattle housed under commercial conditions may provide researchers with a better understanding of how to develop useful and applied research to stimulate advances in the finishing cattle industry. Therefore, our first experiment was completed to outline the practices used by consulting feedlot nutritionists and their clients by summarizing information on cattle management techniques, facility design, commodity use and processing, nutrient formulations, and animal health considerations. The data presented by Galyean and Gleghorn (2001), Vasconcelos and Galyean (2007), and Samuelson et al. (2016a) in feedlot consulting nutritionist surveys has been useful for both the industry and academic communities, to provide a reference point for cattle management techniques and illustrate how the finishing cattle industry has changed over the past 15 years.

Interestingly, the nutritional and management recommendations reported by feedlot nutritionists in the current survey indicate that management practices have remained relatively stable with the exception of alterations in feedstuff preferences and changes due to advances in technology. For example, one of the most glaring changes in diet formulation noted in the results reported by Samuelson et al. (2016a) was a decrease in the concentration of grain included in finishing diets in favor of greater amounts of grain byproducts. This was of particular interest to the authors as anecdotal reports from those in industry suggested that the higher inclusion rate of grain byproducts influenced overall dietary CP concentrations so that cattle were consuming CP in excess of nutrient requirements. When dietary CP is provided in amounts that exceed the requirements for total protein, the surplus must be converted to urea and excreted from the body. Increased ureagenesis may result in greater liver energy expenditure and/or AA catabolism to supply the substrates needed for urea synthesis (Lobley et al., 1995), and therefore could further impact livestock requirements for energy and protein. The feedlot consulting nutritionists surveyed by Samuelson et al. (2016a) recommended maximum CP concentrations of 15.9% of DM for finishing cattle. However, because many of the grain byproducts used in cattle diets contain high concentrations of CP, it is possible that cattle in feedlots are receiving dietary CP in concentrations greater than the maximum recommended by consulting nutritionists and subsequent nutritional needs for dietary CP.

Because orally active βAA for cattle had only recently been approved in the U.S. at the time of the survey completed by Vasconcelos and Galyean (2007), information regarding their use in finishing cattle diets was not reported. This information was added to the survey completed by Samuelson et al. (2016a) along with several other questions in an attempt to gauge the use of non-nutritional feed additives in cattle diets. The nutritionists surveyed reported that approximately 84.8% of their clients were using a βAA during the final phase of the finishing period (Samuelson et al., 2016a). Because the results of the survey reported by Samuelson et al. (2016a) indicate that concentrations of dietary CP in finishing cattle diets have likely increased, greater protein synthesis (such as that achieved through βAA administration) may decrease

the need to dispose of excess dietary CP while also potentially increasing animal performance.

Dietary protein consumed by ruminants can provide sources of both RDP and RUP. When consumed, RDP is available within the rumen for microbial degradation and microbial CP synthesis (Bach et al., 2005). In contrast, RUP escapes the rumen and flows directly to the lower tract for post-ruminal digestion and absorption (Church, 1993). Together, both microbial CP (from RDP) and RUP make up MP that can be used by the animal for maintenance or growth, or excreted from the body in the form of urea when absorbed in excess of requirements (Church, 1993). Increasing protein deposition and therefore overall requirements for net protein likely results in decreased AA catabolism and ureagenesis. Brake et al. (2011) reported that when cattle were fed ZH, a lower proportion of urea-N was recycled to the gut. Therefore, our second experiment was completed to investigate the effects of ZH on cattle performance and carcass merit when diets with increasing concentrations of RDP (supplied as urea) were fed. The study was a 2 × 3 factorial arrangement of either 0 or 75 mg ZH per steer daily added to diets containing 0, 0.5, or 1.0% urea (7.3, 8.4, and 9.7% RDP respectively) for the last 24 d of the finishing period, followed by a 3 d withdrawal for ZH. No interactions between ZH administration and dietary RDP were observed by Samuelson et al. (2016b) for both steer performance and carcass characteristics. This suggests that cattle receiving a βAA do not have greater requirements for RDP than those not receiving a βAA.

In the study completed by Samuelson et al. (2016b), feeding ZH resulted in comparable improvements in both live and carcass performance to those reported in previous studies (Vasconcelos et al., 2008; Holland et al., 2010) where cattle received ZH during the last 28 to 42 d of the finishing period. When fed increasing concentrations of dietary RDP supplied as urea, DMI and ADG decreased linearly, with the greatest decrease observed as urea was increased in the diet from 0.5 to 1.0% (Samuelson et al., 2016b). Cooper et al. (2002) reported that approximately 8.3% RDP is adequate to meet the RDP requirements of finishing cattle consuming steam-flaked corn based diets with no βAA. Because the intermediate diet (0.5% urea) used by Samuelson et al. (2016b) contained approximately 8.4% RDP, it is likely that the 1.0% urea diet (9.7% RDP) provided RDP in excess of the microbial capacity to synthesize microbial CP. Furthermore, because DMI of cattle receiving the 1.0% urea diet restricted dietary energy intake, it is likely that microbial CP synthesis was limited by energy availability which may have resulted in an even greater surplus of RDP. Excess RDP results in increased ammonia supplied to the liver for ureagenesis. Because ammonia may impact satiety signaling and/or meal patterns of livestock (Conrad et al., 1977; Oba and Allen, 2003), it is possible that a greater amount of ruminally availably N from RDP limited DMI in the present study. Therefore, the lower performance observed by Samuelson et al. (2016b) in the high urea diet may have been a result of excess dietary RDP.

In addition to excess dietary RDP, high concentrations of RUP may also contribute to ammonia surplus and subsequent increases in ureagenesis. In ruminants, detoxification of ammonia to urea may occur in response to surplus MP (from rumen microbial CP plus RUP) or high ruminal ammonia concentrations from RDP (Lobley et al., 1995). Previous research (Lobley et al., 1995; Mustvangwa et al., 1997) indicates that a high liver ammonia load results in increased splanchnic deamination and oxidation of AA, and increased liver oxygen consumption. Therefore, it is possible that high concentrations of dietary CP supplied as either RDP or RUP could increase the metabolic costs of ureagenesis. Dunshea et al. (1993) reported that pigs consuming RH had greater ADG as the concentration of CP in the diet was increased above 14.0%. Therefore, increased protein deposition may have the potential to offset needs for urea synthesis and decrease potentially negative effects on animal performance during provision of excess dietary CP. Consequently, the third experiment was conducted to investigate the effects of RH on cattle performance and carcass characteristics when cattle were consuming diets formulated to supply dietary CP (from either primarily RDP or RUP) in excess of nutrient requirements. The study was a 2 × 3 factorial arrangement where cattle received either 0 or 400 mg per heifer daily added to 1 of 3 dietary protein treatments for the last 35 d of the finishing period. The 3 dietary protein treatments were: 13.9% CP, 8.9% RDP, and 5.0% RUP (CON); 20.9% CP, 14.4% RDP, and 6.5% RUP (High RDP), and 20.9% CP, 9.7% RDP, and 11.2% RUP (High RUP). No interactions were observed between RH and dietary protein for any of the response variables measured by Samuelson et al. (2016c). This indicates that providing CP in concentrations greater than those used in typical finishing diets does not improve performance of cattle receiving a βAA. Similarly, research completed by Walker et al. (2006), Samuelson et al. (2016b), and Hales et al. (2016) using lower CP concentrations reported that performance of cattle receiving a βAA was not influenced by providing additional CP in the diet. Taken together, this research also suggests that cattle need for protein degradability are not altered by βAA administration. Cattle receiving RH had greater performance and lower carcass fat than those receiving no RH

(Samuelson et al., 2016c), which agrees with previous research (Avendaño-Reyes et al., 2006; Scramlin et al., 2010). However, Samuelson et al. (2016c) suggests that providing higher concentrations of RH may increase the magnitude of the observed response.

Measurements of live performance such as DMI, ADG, and G:F were not different among the 3 dietary CP treatments (Samuelson et al., 2016c). Generally, increasing dietary CP in cattle diets will result in linear increases in performance until the maximum capacity for protein deposition is reached (Campbell, 1988). This suggests that the control diet (13.9% CP) of Samuelson et al. (2016c) supplied either adequate or greater concentrations of dietary CP than needed for cattle to maximize their growth potential. Regardless of degradability, excess CP did not negatively affect cattle performance (Samuelson et al., 2016c), which indicates that the potential metabolic costs associated with ureagenesis during provision of excess CP are not large enough to impact overall animal performance.

IMPLICATIONS AND FUTURE RESEARCH

It is my hope that the results of this research will be useful for both the scientific and industry communities interested in beef cattle nutrition, particularly those who have interests in nutritional management of cattle during the finishing phase. While the feedlot industry is both dynamic and progressive, a large body of work and experience goes into developing nutritional and management recommendations for feedlot cattle. Therefore, previous successes may play a large role in governing the paradigm that dictates how cattle are fed and managed in the U.S. In particular, a combination of both basic and applied research as well as knowledge of application are important for obtaining the best results.

The results of this study indicate that using βAA as a component of finishing cattle diets does not increase dietary CP requirements above what is typically provided in the feedlot. This suggests that when fed under typical conditions, dietary CP concentrations provide adequate MP for both maintenance and growth, even when protein deposition is increased. Although the results of the second experiment suggest that when supplied as RDP, excess dietary CP may negatively impact DMI and animal performance, these results were not observed in the third experiment when an even greater concentration of dietary CP was fed. Furthermore, excess RUP did not contribute to lower performance in the third experiment. Because the mechanisms that govern protein and energy metabolism are complex, additional research on a molecular/basic level might contribute additional insight into why the results were observed in the present research.

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Effects of maternal nutritional status on nutrient transporter expression and nutrient concentrations in bovine utero-placental tissues and fluids on days 16 to 50 of gestation[1]

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ABSTRACT: We hypothesized that maternal nutrition and day of gestation would impact the mRNA expression of glucose transporters *GLUT1*, *GLUT3*, and *GLUT14*, fructose transporter *GLUT5*, and cationic amino acid transporters *CAT-1*, *CAT-2*, and *CAT-3* as well as reduce the concentration of glucose, fructose and cationic amino acids in bovine utero-placental fluids of beef heifers. Crossbred Angus heifers (n = 49) were bred via AI, assigned to nutritional treatment (CON = 100% of requirements for 0.45 kg/d gain and RES = 60% of CON) and ovariohysterectomized on d 16, 34, or 50 of gestation, or not bred (NB) and ovariohysterectomized on d 16 of the synchronized estrous cycle. The resulting arrangement of treatments was a 2 × 3 factorial + 1. Caruncle (CAR), intercaruncular endometrium (ICAR), and fetal membranes (FM), were obtained from the pregnant uterine horn immediately following ovariohysterectomy. For NB controls, only CAR and ICAR were obtained. Maternal serum, histotroph flushed from the horn ipsilateral to the CL (Histo), allantoic fluid (ALF), and amniotic fluid (AMF) were collected at time of ovariohysterectomy. Allantoic and AMF were only collected on d 34 and 50 due to lack of presence in d 16, and NB controls. Expression of *CAT-2* in ICAR was the only gene in any tissue to demonstrate a day × treatment interaction, being greater ($P = 0.01$) in d 50 CON compared with d 34 CON and d 16 and 50 RES. Expression of nutrient transporters were not influence by nutritional treatment ($P > 0.05$); however, transporters were differentially expressed by day of gestation ($P \leq 0.05$). Fructose concentration in AMF was the only nutrient investigated to demonstrate a day × treatment interaction being greater ($P = 0.04$) in d 34 RES compared with d 50 CON and RES. Glucose concentration in ALF from CON dams was greater ($P = 0.05$) compared with RES. Concentrations of nutrients in maternal and fetal fluids were more affected by day of gestation ($P \leq 0.05$) than nutritional treatment. We interpret these results to indicate that day has a greater influence than a 40% global nutrient restriction on the gene expression of nutrient transporters in bovine utero-placental tissues and the concentration of nutrients in bovine maternal and fetal fluids; however, concentrations of fructose and glucose, which were affected by a day × treatment interaction, and main effect of treatment respectively, merit additional investigation of sugar metabolism and use by the developing fetus.

Key words: arginine, fructose, glucose, maternal nutrition, transporters
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INTRODUCTION

Fetal growth is vulnerable to maternal dietary nutrient deficiencies during the first trimester of gestation (Wu et al., 2004). Maternal undernutrition alters placental growth, reduces amino acid and glucose transport, and increases apoptosis and autophagy (Zhang et al., 2015). Before the establishment of hemotrophic nutrition, the placenta is developing and the fetus begins to utilize increasing quantities of nutrients from histotroph including glucose

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and amino acids (Bazer et al., 2011; Groebner et al., 2011). Additionally, the placenta metabolizes glucose to fructose, yielding fructose as the most abundant hexose sugar in fetal fluids (Kim et al., 2012). Thus, the presence of glucose, fructose and amino acid transporters in the utero-placenta becomes essential to the viability of the conceptus. Therefore, we studied the utero-placental glucose transporters *GLUT1*, *GLUT3*, and *GLUT14* (*SLC2A1*, *SLC2A3*, and *SLC2A14,* respectively) of which *GLUT1* and *GLUT3* are high affinity glucose transporters, and *GLUT14* is a duplicon of *GLUT3*; Fructose transporter *GLUT5* (*SLC2A5*) which is a high affinity fructose transporter; and the amino acid transporters *CAT-1*, *CAT-2*, and *CAT-3* (*SLC7A1*, *SLC7A2*, and *SLC7A3*, respectively*)* whose substrates are cationic amino acids such as arginine. Additionally, we measured the concentrations of glucose, fructose, and arginine in utero-placental fluids. Previous research from Crouse et al. (2016b) identified d 16, 34, and 50 as key dates for expression of transporters during early gestation and thus are the days of gestation investigated within. Therefore, we hypothesized that maternal nutrient restriction and day of gestation would impact the mRNA expression of glucose transporters *GLUT1*, *GLUT3*, and *GLUT14*, fructose transporter *GLUT5*, and cationic amino acid transporters *CAT-1*, *CAT-2*, and *CAT-3* as well as reduce the concentration of glucose, fructose and cationic amino acids in bovine utero-placental fluids of beef heifers.

MATERIALS AND METHODS

All animal procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee (#A14053 and Al6049).

Animals, Housing and Diet

Crossbred Angus heifers were obtained from the Central Grasslands Research and Extension Center (Streeter, ND; n = 49, ~16 mo of age; average initial BW = 325 kg) and housed at the NDSU Animal Nutrition and Physiology Center. Heifers were acclimated to individual bunk feeding (American Calan, Northwood, NH) for two weeks before the beginning of the trial. All heifers were exposed to the 5-d CO-Synch + CIDR estrus synchronization protocol (Bridges et al., 2008). Six heifers were not inseminated to serve as non-bred (NB) controls, but received ovariohysterectomy for tissue and fluid collections on d 16 of the synchronized estrous cycle. The remaining heifers were bred by AI bred to a common sire at 12 h after observed estrus, and were randomly assigned to one of two treatment groups. Control heifers (CON), received 100% of NRC (2000) requirements for 0.45 kg/d gain to reach 80% of mature BW at first calving. Restricted heifers (RES), were placed on a 40% global nutrient restriction which was accomplished by reducing total diet delivery to 60% of the control delivery. The diet was delivered via TMR (48.4% DM, 5.3% CP, 6.8% ash, and 29.4% NDF on a DM basis) and consisted of grass hay, corn silage, alfalfa haylage, grain and mineral mix, and supplemented dried distillers grains with solubles (53.4% NDF, 31.3% CP) in addition to the TMR to meet individual heifers protein requirements. All treated heifers were ovariohysterectomized on either d 16 (CON, n = 7; RES, n = 7), 34 (CON, n = 6; RES, n = 9), or 50 (CON, n = 7; RES, n = 7) of gestation. Thus, experimental design for the pregnancy analysis was a 2 × 3 factorial design.

Tissue Sample Collection and Analysis

Ovariohysterectomy procedures were conducted as described by McLean et al., (2016). Briefly, ovariohysterectomy was conducted as a standing procedure, with a left flank incision. Uterine and ovarian arteries were sutured and ligated, along with sutures being placed along the cervix. The uterus was clamped caudal to the bifurcation, and incised along the clamp, thereby collecting the entire uterine body and horns, along with the attached ovaries. Immediately following ovariohysterectomy, utero-placental tissues (caruncle, CAR; intercaruncular endometrium, ICAR; fetal membranes, FM [chorioallantois, d 16 and 34]; were obtained from the uterine horn ipsilateral to the Corpus Luteum, which was also the horn containing the conceptus when observable, as previously described (Grazul-Bilska et al., 2010, 2011). Once collected, all tissues were snap frozen in liquid nitrogen cooled isopentane (2-Methylbutane; J.T.Baker, center Valley, PA) and stored at -80°C.

Real-time quantitative PCR (qPCR) (Intra-Plate CV = 1.14, Inter-Plate CV = 2.46) was performed on CAR, ICAR, and FM samples to determine mRNA expression of transporters. Glucose and fructose transporters of interest were: GLUT1- facilitative diffusion glucose transporter, which is found in most tissues throughout the body and is ubiquitous across mammalian species; GLUT3- facilitative diffusion high capacity glucose transporter known for neural and placental glucose transport; GLUT5- facilitative diffusion fructose transporter; and GLUT14- duplicon of GLUT3 bearing 95% homology previously isolated in testis and bovine utero-placental tissues. The cationic amino acid transporters of interest were CAT-1, CAT-2, and CAT-3, all of which are facilitated diffusion arginine and lysine transporters. The RNA was extracted and

TABLE 1. Primer sequences used for real-time quantitative reverse-transcription PCR

Gene[1]	Forward primer (5'-3')	Reverse primer (5'-3')	NCBI Gene ID	Source
GLUT1	CGGCTGCCCTGGATGTC	GCCTGGGCCCACTTCAAA	282356	Mattmiller et al., 2011
GLUT3	CAAGTCACAGTGCTAGAGTCTTTC	GGAGAGCTGGAGCATGATAGAGAT	282358	Mattmiller et al., 2011
GLUT5	AGTCTCCTGGCAAACGAAGA	AAGAAGGGCAGGAAGAGGAG	282868	Forde et. al., 2011
GLUT14	TATGCTTTGGAAAAGTGGTCAGGAACC	GATGGAGAAGGAACCGATCATA	538810	Crouse et al., 2016a
CAT-1	CCGATAATCGCCACCTTAACCT	ACCAGGTCCTTCAGGTCGAA	539465	Liao et al., 2007
CAT-2	CTGCAAGTGCCAGGGACCCAC	GGTTGCAGCCCCAGCCAAAGT	538708	Forde et al., 2014
CAT-3	GTAGCCCCAACCCAACTCGGC	TGCTAGGAAGGATCGAGGAGCTGT	539065	Forde et al., 2014
ACTB	TGTCCACCTTCCAGCAGATGT	AGCTCAGTAACAGTCCGCCTAGA	280979	Pan et al., 2013

[1]*GLUT1, GLUT3,* and *GLUT14* (LOC538810)- Glucose transporter solute carrier family 2 member 1, 3, and 14. *GLUT5*- Fructose transporter solute carrier family 2 member 5. *CAT-1*, *CAT-2*, and *CAT-3*- Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3. *ACTB* - β-actin.

purified using Aurum Total RNA Mini Kit (Bio-Rad, Hercules, CA), and cDNA was synthesized utilizing iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Total quantity of RNA was determined using Take3 module of a Synergy H1 Microplate Reader (BioTek, Winooski, VT). Optimal cDNA dilutions were determined by primer validation for each gene and tissue type across days of gestation. Primer sequences were sourced through GenBank (Bethesda, MD; Table 1). Gene expression was quantified using a 7500 Fast, Real-Time PCR System (Applied Biosystems, Grand Island, NY) with SYBR Green Master Mix (Bio-Rad Laboratories, Hercules, CA). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with *ACTB* as a reference gene due to consistency of expression across day of gestation and utero-placental tissue type, compared with other reference genes investigated such as *GAPDH*, *YWHAZ*, *TBP*, and *SDHA*.

Fluid Collection and Analysis

Serum samples were collected via jugular venipuncture at time of ovariohysterectomy using 10 mL serum vacutainer tubes (Becton Dickinson HealthCare, Franklin Lakes, NJ), and centrifuged at 1,500 × g for 30 minutes. Serum was separated from blood constituents and stored at -20°C. Allantoic Fluid (ALF) was collected by isolating the embryo within the uterine horn, and extracting 10 mL of fluid from the chorioallantoic sac using a 22-gauge needle (Medtronic, Minneapolis, MN). Amniotic Fluid (AMF) was collected using a 22-gauge needle (Medtronic) inserted through the amnion with suction applied via syringe after the amniotic sac containing the embryo was visualized; 1 mL of fluid was collected from the d 34 embryo and 10 mL from d 50 embryos. Allantoic and amniotic fluids were only collected on d 34 and 50 of gestation. Once collected, all fluids were snap frozen in liquid nitrogen cooled isopentane and stored in -20°C. Uterine luminal fluid (histotroph) was collected from the uterine horn ipsilateral to the corpus luteum (Histo). Histotroph was collected by flushing the uterine horn ipsilateral to the corpus luteum with 20 mL of 10 mM Tris (pH = 7.2). Recovered Histo was centrifuged at 1,000g for 15, supernatant decanted and immediately snap frozen in 1 mL aliquots in liquid nitrogen for subsequent amino acid analysis (Forde et al., 2014).

Arginine, ornithine, citrulline, and lysine concentrations were determined using ACQUITY UPLC System (Waters Corporation, Milford, MA). For UPLC, 250 µL of fluid was used for plasma, ALF, and AMF. The MassTrac Amino Acid Analysis System for Waters UPLC was used to determine the full profile of amino acids in physiological fluids. Glucose concentrations were determined using Infinity Glucose Hexokinase Liquid Stable Reagent (Fisher Diagnostics, Middletown, VA), and analyzed with a Synergy H1 Microplate Reader (BioTek, Winooski, VT). For glucose determination, 5 µL of fluid was used for plasma, ALF, and AMF with 250µL of reagent (Intraplate CV = 3.04; Across-plate CV = 7.39). Fructose concentrations were determined using EnzyChrom™ Fructose Assay Kit (Bio Assay Systems, Hayward, CA), and analyzed with a Synergy H1 Microplate Reader (BioTek, Winooski, VT). For fructose determination, serum, histotroph, allantoic, and amniotic fluid samples were centrifuged prior to pipetting. Allantoic and amniotic fluid samples were diluted 1:10 prior to assay. For the assay, 20 µL of sample was mixed with 80 µL of working reagent (Intraplate CV = 7.16 Across Plate CV - 12.92).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS version 9.4 (SAS Inst. Inc.) with day, treatment and day × treatment in the model, with individual heifer serving as the experimental unit. For tissues, nonbred CAR, ICAR, and endometrium (whole uterine

endometrium including both caruncles and intercaruncular tissue) samples served as the baseline control for gene expression measurements within CAR, ICAR, and FM, respectively. If no significant interactions were present, main effects of maternal nutrition and day of gestation were reported. Means were separated using the LSMEANS procedure of SAS, and *P*-values ≤ 0.05 were considered significant. Additionally, if no significant interactions were present in tissues as well as serum and Histo, contrasts were conducted incorporating the NB heifers comparing the mRNA expression and nutrient concentrations for NB vs. pregnant heifers, as well as d 16 vs. d 34 and 50 (pre-attachment vs. post-attachment), and day post-attachment comparison (d 34 vs. 50) for each individual gene using the GLM procedure of SAS, with *P*-values ≤ 0.05 being considered different.

RESULTS

CAR

Expression of glucose transporter *GLUT1* was not influence by a day × treatment interaction ($P = 0.23$; Table 2) and was greater ($P < 0.01$) on d 16 (2.89-fold) compared with d 34 and 50 (0.85 and 1.14-fold; SEM 0.31; Table 2). There was no day × treatment interaction for *GLUT3* in CAR ($P = 0.87$) and was greater ($P = 0.03$) on d 50 compared with d 16 (10.38 vs. 2.59 respectively, SEM = 2.86; Table 2). Expression of *GLUT3* in CAR (data previously published and presented here for completeness of data presentation; Crouse et al., 2016a,b) was greater ($P = 0.05$) in pregnant heifers compared with NB controls. Additionally, *GLUT3* was greater ($P = 0.01$) on d 34 and 50 compared with d 16. Fructose transporter *GLUT5* expression was not influenced by a day × treatment interaction ($P = 0.20$) and was greater ($P = 0.02$) on d 34 (44.4-fold) of gestation compared with d 16 and 50 (4.79 and 6.49-fold; SEM = 10.9; Table 2). Additionally, *GLUT5* tended ($P = 0.06$) to be greater in expression on days post-attachment (34 + 50) compared with day pre-attachment (d 16; Table 2). Expression of *GLUT14* (data previously published; Crouse et al., 2016a,b) was not influenced by a day × treatment interaction in CAR ($P = 0.69$) and was greater ($P < 0.05$) on d 50 compared to d 16 and 34 (9.27 vs. 1.53 and 2.65 respectively, SEM = 2.93). As well, *GLUT14* was greater ($P = 0.02$) on d 50 compared with d 34 and tended ($P = 0.07$) to be greater on d 34 and 50 compared with d 16 (Table 6). A day × treatment interaction did not influence the mRNA expression of *CAT-1* in CAR ($P = 0.90$). However, *CAT-1* was greater ($P < 0.01$) on d 34 (5.22-fold) compared with d 16 and 50 (1.47 and 0.51-fold; SEM = 0.73; Table 2). Additionally, expression of *CAT-1* tended ($P = 0.09$) to be greater in expression on days post-attachment compared with day pre-attachment (Table 2). Expression of *CAT-2* in CAR was not influence by a day × treatment interaction ($P = 0.89$), but was greater ($P = 0.02$) on d 34 (14.67-fold) compared with d 16 (4.36-fold; SEM = 2.55) with d 50 (7.78-fold) being equal to both d 16 and 34 of gestation (Table 2). Expression of *CAT-2* tended ($P = 0.06$) to be greater in pregnant heifers compared with non-bred heifers. Additionally, *CAT-2* expression was greater ($P = 0.02$) on days post-attachment compared with pre-attachment (Table 2). Expression of cationic amino acid transporter *CAT-3* was not influenced by a day × treatment interaction ($P = 0.90$) and *CAT-3* tended ($P = 0.06$) to be greater in expression in CON heifers compared to the RES heifers across all days of gestation (2.60 and 1.16-fold greater than NB; SEM = 0.64; Table 2).

ICAR

Expression of *GLUT1* in ICAR was not influenced by a day × treatment interaction ($P = 0.42$) and was greater on d 16 (2.11-fold) compared with d 34 (0.75-fold greater than NB; SEM = 0.28; Table 2). Additionally, *GLUT1* expression was greater ($P < 0.01$) in expression on day pre-attachment compared with days post-attachment, as well as tending ($P = 0.10$) to be greater on d 50 compared with d 34 (Table 2). Expression of *GLUT3* in ICAR (data previously published; Crouse et al., 2016a,b) was not influenced by a day × treatment interaction ($P = 0.19$) and was greatest ($P = 0.02$) on d 50 compared with d 16 and 34 of gestation (3.20 vs. 1.96 and 1.14, respectively, SEM = 0.70; Table 2). Glucose transporter *GLUT3* tended ($P = 0.08$) to be greater in CON heifers in ICAR compared with RES. Fructose transporter *GLUT5* was not influenced by a day × treatment interaction, or a main effect of day or treatment ($P \geq 0.05$; Table 2). In ICAR, *GLUT14* (data previously published; Crouse et al., 2016a,b) was not influenced by a day × treatment interaction ($P = 0.55$) and tended ($P = 0.09$) to be greater on d 16 compared with d 34 (Table 2). Expression of *CAT-1* was not influenced by a day × treatment ($P = 0.89$) and was greater ($P < 0.01$) on d 34 and 50 (14.62 and 11.13-fold, respectively) compared with d 16 (0.58-fold; SEM = 1.84; Table 2). Additionally, average expression of pregnant heifers across all days of gestation was greater ($P < 0.01$) than non-bred. Expression of *CAT-2* was influenced by a day × treatment interaction, being greater ($P = 0.01$) on d 50 CON (7.78-fold) compared with d 16 and 50 RES and d 34 CON (3.10, 3.17, and 2.31-fold, respectively; SEM = 1.43; Table 2). Additionally, d

TABLE 2. Relative gene expression of nutrient transporters *GLUT1*, *GLUT3*, *GLUT5*, *GLUT14*, *CAT-1*, *CAT-2*, and *CAT-3* in caruncular (CAR), intercaruncular (ICAR), and fetal membrane (FM) tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-bred (NB) controls set to 1

				Day of gestation[1]				*P* - value[2]					
Tissue	Gene[3]	Trt[4]		16	34	50	SEM[5]	NB vs. Preg	16 vs. 34 + 50	34 vs. 50	Day	Trt	Day × Trt
CAR	*GLUT1*	CON		2.40	0.93	1.38	0.44	0.21	< 0.01	0.47	< 0.01	0.77	0.23
		RES		3.38	0.76	0.89							
			Day[6]	2.89^{g}	0.85^{h}	1.14^{h}							
	GLUT3	CON		3.60	6.40	10.07	2.86	0.05	0.01	0.13	0.03	0.85	0.87
		RES		1.57	6.55	10.68							
			Day	2.59^{b}	6.47^{ab}	10.38^{a}							
	GLUT5	CON		6.61	22.05	9.07	15.33	0.24	0.06	< 0.01	0.02	0.35	0.20
		RES		2.97	66.74	3.91							
			Day	4.79^{h}	44.40^{g}	6.49^{h}							
	GLUT14	CON		2.62	2.76	11.95	2.93	0.32	0.07	0.02	0.03	0.30	0.69
		RES		0.44	2.55	6.60							
			Day	1.53^{b}	2.65^{b}	9.27^{a}							
	CAT-1	CON		1.08	5.24	0.53	1.03	0.21	0.09	< 0.01	< 0.01	0.78	0.90
		RES		1.85	5.20	0.49							
			Day	1.47^{h}	5.22^{g}	0.51^{h}							
	CAT-2	CON		5.76	14.37	7.98	3.63	0.06	0.02	0.04	0.02	0.77	0.89
		RES		2.95	14.97	7.58							
			Day	4.36^{h}	14.67^{g}	7.78^{gh}							
	CAT-3	CON		1.29	2.23	4.28	1.09	0.44	0.24	0.11	0.20	0.06	0.90
		RES		0.45	0.99	2.05							
			Day	0.87	1.61	3.16							
ICAR	*GLUT1*	CON		2.44	0.63	1.77	0.39	0.43	< 0.01	0.10	< 0.01	0.26	0.42
		RES		1.77	0.87	1.08							
			Day[7]	2.11^{g}	0.75^{h}	1.43^{gh}							
	GLUT3	CON		2.80	0.90	4.13	0.70	0.27	0.71	0.01	0.02	0.08	0.19
		RES		1.13	1.39	2.27							
			Day	1.96^{b}	1.14^{b}	3.20^{a}							
	GLUT5	CON		1.45	3.60	6.11	3.30	0.36	0.58	0.97	0.85	0.66	0.31
		RES		3.59	3.82	1.20							
			Day	2.52	3.71	3.66							
	GLUT14	CON		6.74	2.48	4.78	1.50	0.27	0.36	0.29	0.09	0.33	0.55
		RES		5.14	3.04	2.19							
			Day	5.94	2.76	3.48							
	CAT-1	CON		0.65	13.86	9.94	2.59	< 0.01	< 0.01	0.13	< 0.01	0.56	0.89
		RES		0.51	15.37	12.33							
			Day	0.58^{h}	14.62^{g}	11.13^{g}							
	CAT-2	CON		6.83^{ab}	2.31^{c}	7.78^{a}	1.43	-	-	-	0.22	0.68	0.01
		RES		3.10^{bc}	6.08^{abc}	3.17^{bc}							
			Day	4.97	4.19	5.48							
	CAT-3	CON		9.69	1.55	5.53	1.89	0.09	0.12	0.27	0.13	0.45	0.09
		RES		3.87	4.25	5.05							
			Day	6.78	2.90	5.29							

Cont'd.

16 CON (6.83-fold) was intermediate and greater ($P < 0.05$) compared with d 34 CON (2.31-fold; SEM = 1.43; Table 2). Expression of *CAT-3* tended ($P = 0.09$) to be influenced by a day × treatment interaction (Table 2). Additionally, *CAT-3* expression tended ($P = 0.09$) to be greater in pregnant heifers compared with non-bred heifers (Table 2).

TABLE 2. Continued.

Tissue	Gene[3]	Trt[4]		Day of gestation[1] 16	34	50	SEM[5]	*P* - value[2] NB vs. Preg	16 vs. 34 + 50	34 vs. 50	Day	Trt	Day × Trt
FM	*GLUT1*	CON		0.11	0.26	0.26	0.08	-	0.14	0.94	0.35	0.61	0.90
		RES		0.19	0.27	0.27							
			Day[7]	0.15	0.26	0.27							
	GLUT3	CON		0.63	0.30	0.36	0.15	-	0.02	0.36	0.04	0.12	0.33
		RES		0.90	0.64	0.34							
			Day	0.78[a]	0.47[ab]	0.35[b]							
	GLUT5	CON		59.78	43.59	33.40	19.54	-	0.01	0.12	0.04	0.52	0.44
		RES		100.57	46.63	21.38							
			Day	80.17[g]	45.11[gh]	27.39[h]							
	GLUT14	CON		0.60	0.79	1.51	0.83	-	0.74	0.38	0.62	0.76	0.94
		RES		1.17	0.93	1.44							
			Day	0.88	1.86	1.48							
	CAT-1	CON		0.04	0.22	0.19	0.04	-	< 0.01	0.24	< 0.01	0.67	0.99
		RES		0.05	0.24	0.20							
			Day[6]	0.05[h]	0.23[g]	0.19[g]							
	CAT-2	CON		0.42	0.84	1.01	0.28	-	0.04	0.55	0.10	0.82	0.47
		RES		0.24	1.16	0.72							
			Day	0.33	1.00	0.86							
	CAT-3	CON		0.08	1.33	1.32	1.37	-	0.84	0.47	0.76	0.30	0.52
		RES		2.38	0.93	2.20							
			Day	1.23	1.13	1.76							

[1]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NB level of expression set to 1.

[2]Probability values for effect of day, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NB vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.

[3]Gene = *GLUT1, GLUT3,* and *GLUT14*- Glucose transporter solute carrier family 2 members 1, 3, and 14. *GLUT5*- Fructose transporter solute carrier family 2 member 5. *CAT-1*, *CAT-2*, and *CAT-3*- Cationic amino acid transporters of arginine and lysine, solute carrier family 7 members 1, 2, and 3.

[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 0.45 kg daily. RES = Heifers restricted to 60% of CON diet

[5]Average SEM was used within gene. NB n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7.

[6]Mean level of expression across treatment within day and gene of interest.

[a-c]Interactive Means within gene without a common superscript differ ($P \leq 0.05$).

[g,h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

FM

Glucose transporter *GLUT1* expression was not influenced by a day × treatment interaction ($P = 0.90$), nor a day or treatment main effect ($P = 0.35$ and $P = 0.61$, respectively). Expression of *GLUT3* in FM (data previously published; Crouse et al., 2016a,b) was not influence by a day × treatment interaction ($P = 0.33$) and was greater ($P = 0.04$) on d 16 compared with d 50. Expression of *GLUT5* was not influenced by a day × treatment interaction ($P = 0.44$), however was greater ($P = 0.04$) on d 16 (80.17-fold) compared with d 50 (27.39-fold; SEM = 13.8; Table 2). Additionally, d 34 (45.11-fold) was intermediate and equal ($P = 0.12$) to both d 16 and 34 (80.17 and 27.39-fold, respectively; SEM = 13.8; Table 2). Contrast statements for *GLUT5* revealed that expression was greater on days post-attachment than d pre-attachment ($P = 0.01$). Expression of *GLUT14* (data previously published; Crouse et al., 2016a,b) in FM was not influence by a day × treatment interaction, or main effects of day of gestation or treatment ($P > 0.55$). Arginine and lysine transporter *CAT-1* was not influenced by a day × treatment interaction ($P = 0.99$) and was greater ($P < 0.01$) on d 34 and 50 (0.23 and 0.19-fold) compared with d 16 (0.05-fold; SEM = 0.03; Table 2). Expression of *CAT-2* was not influenced by a day × treatment interaction ($P = 0.47$) and tended ($P = 0.10$) to be greater on d 34 (1.00-fold) compared with d 16 of gestation (0.33-fold; SEM = 0.20; Table 2). Cationic amino acid transporter *CAT-3* was not influence by a day × treatment interaction ($P = 0.52$) nor a main effect of day or treatment ($P = 0.76$ and $P = 0.30$, respectively; Table 2).

Serum

No nutrients measured in serum were influenced by a day × treatment interaction or a main effect of maternal nutritional treatment ($P > 0.05$; Table 3). Glucose concentration in serum was greater ($P =$

TABLE 3. Concentrations of glucose (mM), fructose (mM), and arginine (μmol/L) in serum, histotroph (Histo), allantoic (ALF), and amniotic (AMF) fluid samples as influenced by dietary treatment in non-bred (NB) heifers, and from days 16 to 50 of gestation.

				Day of Gestation[1]					*P* - values[2]					
Fluid	Item[3]	Trt[4]		NB	16	34	50	SEM[5]	Day	Trt	Day × Trt	NB vs. Preg.	16 vs. 34 + 50	34 vs. 50
Serum	Gluc	CON		4.76	4.09	4.31	3.73	0.21	0.04	0.29	0.16	< 0.01	0.04	0.15
		RES		-	4.30	3.71	3.57							
			d[6]	4.76	4.20[g]	4.02[g]	3.65[h]							
	Fruc	CON		0.17	0.08	0.07	0.07	0.01	0.97	0.35	0.82	0.02	0.35	0.20
		RES		-	0.08	0.09	0.09							
			d	0.17	0.08	0.08	0.08							
	Arg	CON		144.3	166.9	178.1	207.4	13.11	0.01	0.46	0.98	0.01	0.02	< 0.01
		RES		-	161.1	167.7	199.8							
			d	144.3	164.0[h]	172.9[h]	203.6[g]							
Histo	Gluc	CON		2.07	3.95	3.92	3.49	0.55	0.68	0.74	0.35	0.03	0.40	0.82
		RES		-	2.93	3.87	4.10							
			d	2.07	3.44	3.90	3.80							
	Fruc	CON		0.17	0.12	0.58	1.03	0.31	< 0.01	0.27	0.65	0.09	< 0.01	0.02
		RES		-	0.23	0.69	1.64							
			d	0.17	0.18[h]	0.63[h]	1.34[g]							
	Arg	CON		113.4	151.4	192.2	27.16	47.62	0.14	0.88	0.30	0.25	0.63	0.05
		RES		-	124.9	144.4	118.7							
			d	113.4	138.2	168.3	72.90							
ALF	Gluc	CON		-	-	1.88	1.39	0.17	0.05	0.07	0.45	-	-	-
		RES		-	-	1.42	1.20							
			d	-	-	1.64[g]	1.29[h]							
	Fruc	CON		-	-	5.53	5.07	0.76	0.90	0.44	0.64	-	-	-
		RES		-	-	4.56	4.83							
			d	-	-	5.04	4.95							
	Arg	CON		-	-	620.9	456.6	51.31	0.11	0.37	0.23	-	-	-
		RES		-	-	498.1	475.3							
			d	-	-	559.5	466.0							
AMF	Gluc	CON		-	-	1.85	1.74	0.22	0.34	0.05	0.16	-	-	-
		RES		-	-	1.08	1.61							
			d	-	-	1.46	1.68							
	Fruc	CON		-	-	3.30[ab]	2.57[b]	0.29	< 0.01	0.21	0.04	-	-	-
		RES		-	-	3.56[a]	1.55[c]							
			d	-	-	3.43	2.06							
	Arg	CON		-	-	203.6	253.6	24.24	0.04	0.63	0.88	-	-	-
		RES		-	-	212.1	269.4							
			d	-	-	207.8[h]	261.5[g]							

0.04) on d 16 and 34 of gestation (4.20 and 4.02 mM, respectively) compared with d 50 (3.65 mM; SEM = 0.15; Table 3). Additionally, concentration of glucose was greater ($P < 0.01$) in NB heifers (4.76 mM) compared with their pregnant counterparts (3.93 mM; Table 2). When comparing pre-attachment vs. post-attachment (d 16 vs. 34 + 50) glucose concentration in maternal serum, pre-attachment heifers had greater ($P = 0.01$; SEM = 0.16; 4.20 mM) concentration of glucose compared with heifers in the post attachment days of gestation (3.84 mM; SEM = 0.16; Table 3). Serum concentration of fructose was greater ($P = 0.01$) in NB heifers compared with pregnant heifers (0.17 vs. 0.08, respectively; SEM = 0.01; Table 3). Arginine concentration was greater ($P = 0.01$) on d 50 (203.6 μmol/L) compared with d 16 and 34 (164.0 and 172.9 μmol/L, respectively; SEM = 9.3; Table 3). Concentration of arginine was greater ($P = 0.03$) in pregnant heifers compared with NB heifers (180.2 μmol/L vs. 144.3 μmol/L, respectively; SEM = 9.6; Table 3). Arginine concentration in heifer serum was greater ($P = 0.02$) on days post-attachment compared with pre-attachment (180.2 μmol/L vs. 164.0 μmol/L, respectively; SEM = 8.5; Table 3).

Histotroph

No nutrients in Histo were influenced by a day × treatment interactions ($P > 0.05$), or a main effect of maternal nutritional treatment ($P > 0.05$; Table 3). Glucose concentrations were greater ($P = 0.03$) in luminal flushings of pregnant heifers compared with NB heifers (3.71 mM vs. 2.07 mM, respectively; SEM = 0.46; Table 3). Fructose concentrations in Histo were greater ($P < 0.01$) on d 50 of gestation (1.34 mM) compared with d 16 and 34 (0.18 mM and 0.63 mM, respectively; SEM = 0.22; Table 3). Pregnant heifers tended ($P = 0.09$) to have greater concentrations of fructose in luminal flushings from the luteal horn compared to NB heifers (Table 3). When comparing pre-attachment vs. post-attachment fructose concentrations, post-attachment concentrations of fructose in Histo were greater (0.99 mM; $P < 0.01$) compared with pre-attachment concentrations (0.18 mM; Table 3). Arginine concentrations in Histo were not influenced by day of early pregnancy; however, comparison of days post-attachment determined that concentrations of arginine on d 34 of gestation are greater ($P = 0.05$) compared with d 50 (114.5 vs. 72.7, respectively; SEM = 67.5; Table 2).

Allantoic Fluid

No nutrients in ALF were influenced by a day × treatment interaction ($P > 0.05$). Glucose concentration was greater ($P = 0.05$) on d 34 of gestation, compared with d 50 (1.64 vs. 1.29 mM; SEM = 0.12; Table 3). Additionally, concentration of glucose in ALF tended ($P = 0.07$) to be greater in control vs. restricted heifers (1.63 mM vs. 1.31 mM, respectively; SEM = 0.12; Table 3). Concentrations of fructose and arginine were not influenced by a main effect of day of gestation, or treatment ($P > 0.05$; Table 3).

Amniotic Fluid

Fructose concentration in AMF was influence by a day × treatment interactions, being greater ($P = 0.04$) on d 34 RES (3.56 mM), compared with d 50 CON and RES (2.57 mM and 1.55 mM, respectively). Additionally, d 34 RES and d 50 CON (3.30 mM and 2.57 mM, respectively) were greater ($P \leq 0.05$) compared with d 50 RES (1.55 mM; SEM = 0.29; Table 3). Concentration of glucose was greater ($P = 0.05$) in CON compared with RES heifers (1.79 vs. 1.34 mM; SEM = 0.16; Table 3). Arginine concentration was greater ($P = 0.04$) on d 50 of gestation compared with d 34 (261.49 μmol/L vs. 207.83 μmol/L; SEM = 17.29; Table 3).

DISCUSSION

In keeping with our hypothesis, we determined that *CAT-2* in ICAR was the only gene in any tissue we studied to be influenced by a day × treatment interaction, and that the expression of glucose, fructose, and cationic amino acid transporters in the bovine uteroplacenta are affected more so by day of gestation than a 40% global maternal nutritional restriction up to d 50 of gestation. Additionally, the concentration of fructose in ALF was the only nutrient in any fluid studied to be influenced by a day × treatment interaction, and except for glucose in ALF and AMF, glucose, fructose, and arginine concentration are affected more so by day of gestation than a 40% global maternal nutritional restriction up to d 50 of gestation.

Glucose, fructose, and amino acids, specifically arginine, are crucial for proper energy metabolism and growth, and are key regulators of mTOR (mammalian target of rapamycin), which is linked to angiogenesis and cell proliferation, causing increased fetal growth and mitigating apoptosis (Tan and Miyamoto, 2016; Wang et al., 2016). Previous data from Crouse et al., (2016b) evaluating the gene expression of glucose transporters *GLUT1* and *GLUT3* as well as cationic amino acid transporters *CAT-1*, *CAT-2*, and *CAT-3* on d 16, 22, 28, 34, 40, and 50 of gestation in CAR, ICAR, and FM found similar results across day of gestation as reported here. The detection of *GLUT14*, a duplicon of *GLUT3* (Wu and Freeze, 2002) in bovine utero-placental tissues is novel, and merits further work (Crouse et al., 2016a).

Before the establishment of transplacental exchange, nutrient transporters are the only method of supplying nutrients to the conceptus. During the first 50 d of gestation, fetal nutrients from the maternal system are provided through secretions from the uterine glands collectively known as histotroph (Bazer et al., 2011). Histotroph, also known as uterine milk (Bonnet, 1882), is comprised of nutrients, including amino acids, glucose, fructose, and other substances (Bazer et al., 2011). Glucose and arginine concentrations in Histo reported herein are similar in comparison to previous data in the ovine and bovine, in which concentrations of glucose and arginine increased through the first 20 d of gestation (Forde et al., 2014; Gao et al., 2009). Concentrations of fructose in allantoic fluid were nearly 5-fold greater in comparison to glucose, which was to be expected due to the metabolism of glucose to fructose by the placenta (Kim et al., 2012). The differences seen in the concentrations of glucose and fructose in fetal fluids due to day of gestation and nutritional treatment suggest either modified nutrient transport, or use of the substrates by the fetus. These changes merit further work on the functional roles of glucose and fructose to the nutrient restricted fetus.

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Impacts of stocking density on growth and puberty attainment of replacement beef heifers

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ABSTRACT: Sixty Angus x Hereford heifers were ranked by age and BW (210 ± 2 d and 220 ± 2 kg, respectively) on d 0, and assigned to: a) 1 of 3 drylot pens (10 × 14 m pens; 10 heifers/pen) resulting in a stocking density of 14 m^2/heifer (HIDENS; n = 3), or b) 1 of 3 pastures (25-ha pastures; 10 heifers/pasture), resulting in a stocking density of 25,000 m^2/heifer (LOWDENS; n = 3). Pastures utilized were harvested for hay prior to the beginning of the experiment, and negligible forage was available for grazing to LOWDENS heifers throughout the experiment (d 0 to 182). Heifers received the same diet during the experiment, which averaged (DM basis) 4.0 kg/heifer daily of alfalfa-grass hay and 3.0 kg/heifer daily of a corn-based concentrate. Heifer shrunk BW was recorded after 16 h of feed and water withdrawal on d -3 and d 183 for ADG calculation. On d 0, heifers were fitted with a pedometer fixed behind their right shoulder. Each week pedometer results were recorded and blood samples were collected for puberty evaluation via plasma progesterone. Plasma samples collected on d 0, 28, 56, 84, 112, 140, 161, and 182 were also analyzed for concentrations of cortisol and IGF-I. On d 28, 102, and 175, blood samples were also collected for RNA isolation and analysis of heat shock protein (HSP) 70 and HSP72 mRNA expression. On d 0, 49, 98, 147, and 182, hair samples were collected from the tail switch for analysis of hair cortisol concentrations. No treatment effects were detected (P = 0.66) for heifer BW and ADG. Heifers from LOWDENS had more (P < 0.01) steps/week compared with HIDENS. Heifers from LOWDENS had greater (P = 0.05) mRNA expression of HSP72, and tended (P = 0.10) to have greater mRNA expression of HSP70 compared with HIDENS. Plasma concentrations of cortisol and IGF-I were often greater (P ≤ 0.05) in LOWDENS vs. HIDENS heifers (treatment × day interaction; P < 0.01). Hair cortisol concentrations were greater (P < 0.01) for HIDENS vs. LOWDENS heifers beginning on d 98 (treatment × day interaction; P < 0.01). Heifers from HIDENS experienced delayed puberty attainment and had less (P < 0.01) proportion of pubertal heifers on d 182 compared with LOWDENS (treatment × day interaction; P < 0.01). Altogether, HIDENS negatively impacted heifer stress-related and physiological responses, and delayed puberty attainment compared with LOWDENS.

Key words: Beef heifers, growth, puberty, stocking density
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INTRODUCTION

Public scrutiny on beef production systems is growing rapidly, and cattle welfare is one of the main targets for attention (Grandin, 2014). Cattle producers are challenged with improving production efficiency while fostering animal well-being. Hence, management that increase beef cattle productivity and promote animal welfare are warranted to enhance profitability in beef cattle systems, address the current and projected increases in beef demand, and satisfy industry and public requirements for proper animal care. Stocking density is one example of management that may impact welfare and productive efficiency in cattle operations. In U.S. spring-calving cow-calf herds, replacement heifers are weaned in the fall and exposed to their first breeding season the following spring. Hence, these heifers are commonly reared in drylot systems to facilitate feeding and management

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during the fall and winter. However, rearing cattle in areas with elevated stocking density is known to stimulate stress reactions (Grandin, 2014), while acute and chronic stress directly impairs reproductive function in beef cattle (Dobson and Smith, 2000). Accordingly, Petersen et al. (2014) reported that heifers developed in drylots (11 m^2/heifer) gained more BW but had increased heart rate and rested less compared with contemporary heifers reared on native range (7,400 m^2/heifer). Mulliniks et al. (2013) also indicated that heifers reared in drylots had greater ADG, but reduced pregnancy rates compared with cohorts reared on range pastures.

Based on this information, we hypothesized that elevated stocking density impairs welfare and reproductive development in beef heifers. To test our hypothesis, this experiment compared growth, physical activity, stress-related and physiological responses, and puberty attainment in heifers reared on high (drylots) or low (pastures) stocking densities from weaning until the start of their first breeding season.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University - Eastern Agricultural Research Center (Burns, OR) from September 2015 until March 2016 (d 0 to 182). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (#4757).

ANIMALS AND TREATMENTS

On day 0 of the experiment, 60 Angus x Hereford heifers were ranked by age and BW (initial age= 210±2d; initial BW= 220±2kg) and allocated to: a) 1 of 3 drylot pens (10x14 m pens; 10 heifers/pen), resulting in a stocking density of 14 m^2/heifer (HIDENS; n = 3), or b) 1 of 3 pastures (25-ha pastures; 10 heifers/pasture), resulting in a stocking density of 25,000 m^2/heifer (LOWDENS; n = 3). Pasture and drylot pens were located approximately 800 m and 80 m, respectively, from the handling facility where cattle were processed during the present experiment. Treatments were designed to represent stocking densities of drylot- or pasture-based heifer development programs of commercial cow-calf operations. In addition, HIDENS heifers were exposed to the stocking density recommended for growing cattle reared in drylot systems (Albin and Thompson, 1990). All pastures utilized herein were harvested for hay prior to the beginning of this experiment, and negligible forage was available for grazing to LOWDENS heifers throughout the experimental period. Therefore, all heifers received the same limited-fed diet consisting of 5 kg of alfalfa hay and 3.5 kg of corn per heifer daily, in addition to ad libitum access to water and a commercial mineral and vitamin mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID). Hay was offered separated from concentrate, and the entire diet was completely consumed within 24 h after being offered.

Sampling

Heifer shrunk BW was recorded after 16 h of feed and water withdrawal on d -3 and d 183 for ADG calculation. Heifer temperament was assessed via chute score, exit velocity, and overall temperament score as described by Cooke et al. (2009) on d 0 and 182. On d 0, heifers were also fitted with a pedometer (HJ-321; Omron Healthcare, Inc., Bannockburn, IL) capable of storing daily data for 7 consecutive days placed inside a polyester patch (Heat Watch II; Cow Chips, LLC, Manalapan, NJ) fixed behind their right shoulder to assess physical activity.

Each week during the experiment (day 0 to 182), heifer full BW and pedometer results were recorded, and blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with 158 USP units of freeze-dried sodium heparin for plasma collection. All blood samples were placed immediately on ice, centrifuged (2,500 × g for 30 min; 4°C) for plasma harvest and stored at -80°C.

Plasma progesterone, cortisol, and IGF-1 concentrations were analyzed using a chemiluminescent enzyme immunoassay (Immulite 1000: Siemens Medical Solutions Diagnostics, Los Angeles, CA). The intra- and inter-assay CV were, respectively, 5.1 and 5.8 for progesterone, 4.8 and 7.0% for cortisol, and 7.0 and 4.2% for IGF-1. Heifers were considered pubertal once plasma progesterone concentrations were ≥ 1.0 ng/mL, followed by a cyclic pattern of plasma progesterone < and ≥ 1.0 ng/mL suggestive of normal estrous cycles (Day et al., 1984). Puberty attainment was declared at the second week of elevated progesterone.

On d 28, 102, and 175, blood samples were also collected via jugular venipuncture into PAXgene tubes (BD Diagnostics, Sparks, MD) for subsequent RNA isolation and analysis of heat shock protein (HSP) 70, HSP72 according to procedures described by Rodrigues et al. (2015) Total RNA was extracted from blood samples using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA).

On d 0, 49, 98, 147, and 182, hair samples were collected from the tail switch for analysis of hair cortisol concentrations. Hair was collected using scissors

as close to the skin as possible, and the hair material closest to the skin (2.5 cm of length, 300 mg of weight) was stored at -80°C until processed for cortisol extraction according to procedures described by Moya et al. (2013). Upon extraction, samples were reconstituted in 100 μL of the PBS supplied with a salivary cortisol ELISA kit (Salimetrics Expanded Range, High Sensitivity 1-E3002, State College, PA), and stored at -80°C. Samples were then analyzed for cortisol concentrations using the aforementioned ELISA kit, intra- and inter-assay CV were, respectively, 5.8 and 7.3%.

Statistical Analysis

All data were analyzed using pen or pasture (3 replications/treatment) as experimental unit, with the MIXED or GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC, USA) for quantitative and binary data, respectively, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. All data were analyzed using replication(treatment) and heifer(replication) as random effects. The model statement used for ADG, initial and final BW, as well as heifer BW and age at puberty contained the effects of treatment. The model statement for puberty attainment, physical activity, temperament and physiological variables contained the effects of treatment, day, and the treatment × day interaction. The specified term used in the repeated statement was day, the subject was heifer(replication), and the covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to effect of treatment if no interactions are significant, or according to the highest order interaction detected.

RESULTS AND DISCUSSION

A treatment effect was detected ($P < 0.01$) for physical activity, given that LOWDENS heifers had more steps/week compared with HIDENS heifers throughout the experiment (Table 1). This outcome can be attributed to the greater area that LOWDENS heifers had available. Others have also reported greater physical activity in heifers reared on pasture compared with drylot cohorts (Petersen et al., 2014). However, elevated physical activity may increase maintenance requirements and reduce growth rates in cattle (Cooke et al., 2009), whereas no treatment differences were detected for heifer BW and ADG during the experimental period (Table 1).

TABLE 1. Growth parameters, activity and temperament variables, and blood mRNA expression of heat shock proteins (HSP) in heifers reared in low stocking density (25,000 m^2/heifer; LOWDENS, n = 3) or high stocking density (14 m^2/heifer; HIDENS, n = 3)[1]

Item	HIDENS	LOWDENS	SEM	*P* =
Growth parameters				
Initial BW, kg	211	212	3	0.78
Final BW, kg	355	358	5	0.70
ADG[2], kg/d	0.77	0.78	0.02	0.66
Activity				
Steps/week[3]	19,839	3,147	628	< 0.01
Temperament variables[4]				
Chute score	1.91	1.84	0.09	0.58
Exit velocity, m/s	2.15	1.97	0.16	0.43
Temperament score	2.52	2.37	0.14	0.47
HSP mRNA expression[5]				
HSP70	3.80	2.40	0.48	0.10
HSP72	3.52	2.77	0.20	0.05

[1]From d 0 to 182, HIDENS heifers were reared in 1 of 3 drylot pens (10 x 14 m pens; 10 heifers/pen) and LOWDENS heifers were reared in 1 of 3 pastures (25-ha pastures; 10 heifers/pasture).

[2]Calculated using initial (d -3) and final (d 183) shrunk BW, recorded after 16 h of feed and water withdrawal.

[3]Based on pedometers (HJ-321; Omron Healthcare, Inc., Bannockburn, IL) assessed every 7 d during the experimental period.

[4]According to the techniques described by Cooke et al. (2009), and evaluated on d 0 and 182 of the experiment.

[5]Samples collected on d 28, 102, and 175 of the experiment, processed and evaluated for mRNA expression according to Rodrigues et al. (2015).

Treatment × day interactions were detected ($P < 0.01$) for plasma concentrations of cortisol and IGF-I (Figure 1). Plasma cortisol concentrations were greater in LOWDENS vs. HIDENS heifers on d 84, 140, 161, and 182 of the experiment. Plasma IGF-I concentrations were greater in LOWDENS vs. HIDENS heifers on d 84 and 140 but greater in HIDENS vs. LOWDENS heifers on d 161, which also does not corroborate with similar dietary management and ADG among treatments. Nevertheless, plasma concentrations of IGF-I and cortisol are promptly increased in response to physical activity (Raastad et al., 2000). Hence, treatment effects for plasma cortisol and IGF-I concentrations can be attributed, at least partially, to the additional activity of gathering and bringing the LOWDENS heifers from pasture to the handling facility, whereas HIDENS heifers were grouped in drylot pens adjacent to the handling facility. Yet, treatment differences for plasma IGF-I and cortisol concentrations were inconsistently detected during the experimental period, which cannot be fully explained by the previous rationale or any other variables evaluated herein.

A treatment × day interaction was detected ($P < 0.01$) for hair cortisol concentrations, which were great-

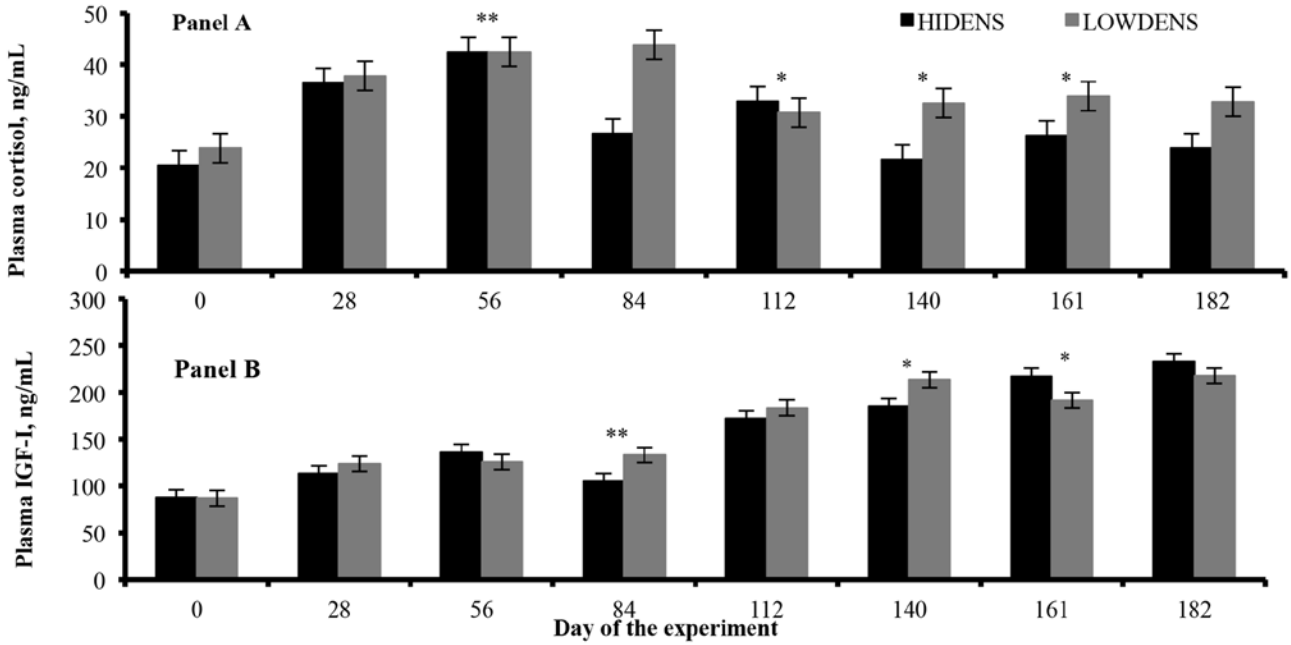

Figure 1. Plasma concentrations of cortisol (Panel A) and IGF-I (Panel B) from heifers reared in low stocking density (25,000 m^2/heifer; LOWDENS) or high stocking density (14 m^2/heifer; HIDENS) from d 0 to 182 the experiment. A treatment × day interaction was detected ($P < 0.01$). Within days, * $P \leq 0.05$, ** $P \leq 0.01$.

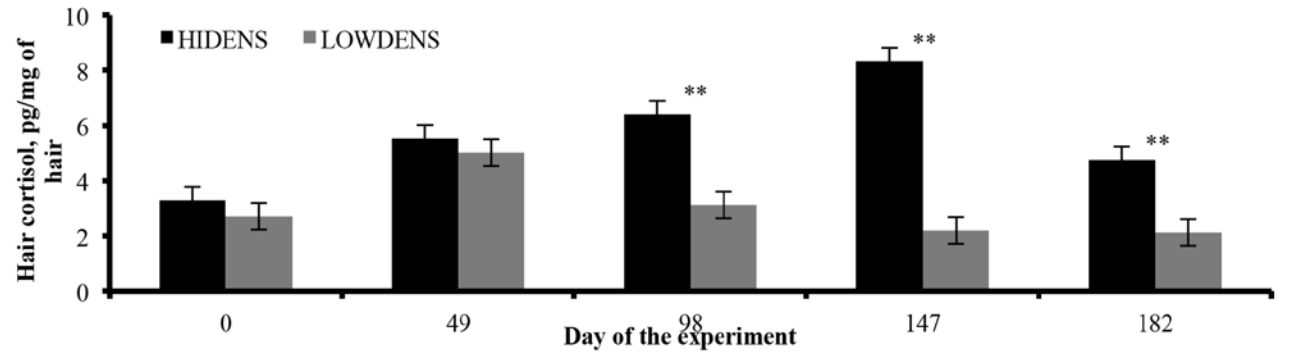

Figure 2. Cortisol concentrations in tail switch hair from heifers reared in low stocking density (25,000 m^2/heifer; LOWDENS) or high stocking density (14 m^2/heifer; HIDENS) from d 0 to 182 the experiment. A treatment × day interaction was detected ($P < 0.01$). Within days, ** $P \leq 0.01$.

er for HIDENS vs. LOWDENS heifers on d 98, 147, and 182 (Figure 2). Cortisol concentration in hair from the tail switch has been recently validated as biomarker of chronic stress in cattle (Burnett et al., 2014), given that cortisol is gradually accumulated in the emerging tail hair and its concentration represents long-term adrenocortical activity (Moya et al., 2013). Hence, treatment differences detected for this variable support our hypothesis that chronic stress and adrenocortical activity were indeed greater in HIDENS compared with LOWDENS heifers. Such outcomes were only noted beginning on d 98 of the experiment, which might be associated with the time required for elevated stocking density to be perceived as a stressor by HIDENS heifers, as well as the time required for hair with elevated cortisol concentration to cross the skin line and become available for collection (Burnett et al., 2014). Treatment effects on hair cortisol concentrations may also help explain the similar ADG among HIDENS and LOWDENS heifers. The greater chronic stress experienced by HIDENS heifers during the experiment may have increased their basal metabolism and maintenance requirements to the same level that physical activity increased these parameters in LOWDENS heifers (NRC, 2000; Petersen et al., 2014).

Heifers from the LOWDENS group had greater (P = 0.05) mRNA expression of HSP72, and tended (P = 0.10) to have greater mRNA expression of HSP70 compared with HIDENS heifers during the experiment (Table 1). Expression of HSP in blood cells can also

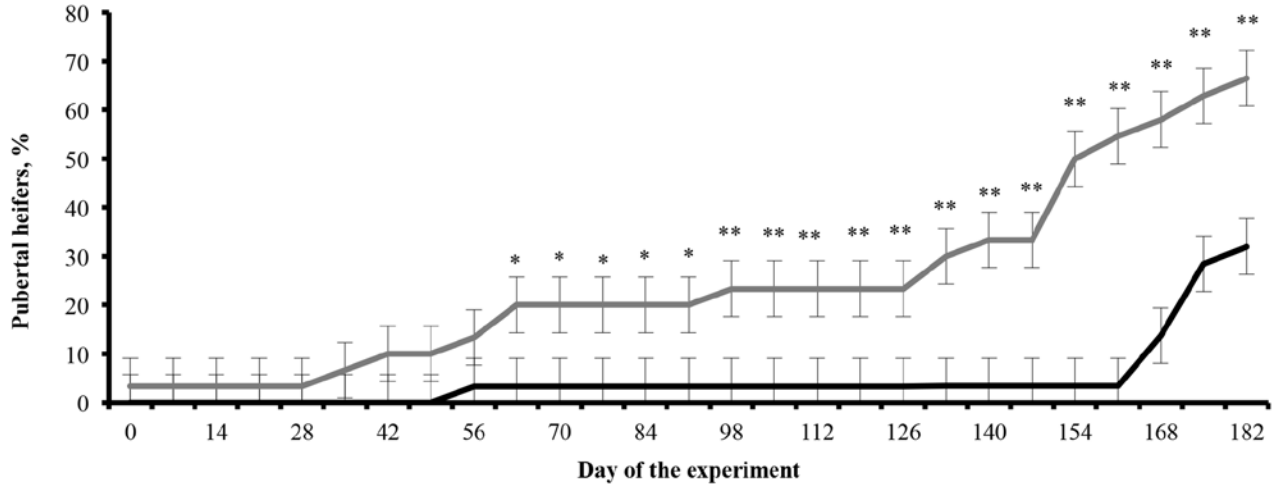

Figure 3. Puberty attainment in heifers reared in low stocking density (25,000 m^2/heifer; LOWDENS) or high stocking density (14 m^2/heifer; HIDENS) from d 0 to 182 the experiment. A treatment × day interaction was detected ($P < 0.01$). Within days, ** $P \leq 0.01$.

be used as a diagnostic marker of stress, given that HSP are rapidly synthesized when cells are exposed to a variety of stressors (Welch, 1992). Hence, treatment effects of blood mRNA expression of HSP70 and HSP72 do not corroborate with our hypothesis and treatment effects detected for hair cortisol concentrations. Nevertheless, HSP mRNA expression may not be directly regulated by elevated circulating cortisol, given that exogenous cortisol administration to fish did not increase mRNA expression of hepatic HSP70 (Basu et al., 2001). Conversely, exercise has been shown to stimulate mRNA expression and circulating concentrations of these HSP in rodents and humans (Milne and Noble, 2002). Hence, treatment effects detected for mRNA expression of HSP70 and HSP72 should be attributed to the greater physical activity of LOWDENS vs. HIDENS heifers, either on a daily basis according to differences in stocking rate and steps/week (Table 1), or during gathering for weekly samplings corroborating with plasma cortisol and IGF-I outcomes (Figure 1).

Rearing cattle in intensive systems, such as drylot with elevated stocking density, results in increased human-animal interaction, which has been shown to impact cattle temperament and subsequent productivity (Cooke et al., 2014). However, LOWDENS and HIDENS heifers had similar ($P \geq 0.26$) chute score, exit velocity, and overall temperament score during the experiment (Table 1), suggesting that treatments evaluated herein were not sufficient to impact heifer temperament variables.

A treatment x day interaction was detected ($P < 0.01$) for puberty attainment. In cattle, age at puberty is highly determined by BW and growth rate (Schillo et al., 1992); however, HIDENS heifers experienced delayed puberty attainment compared with LOWDENS heifers (Figure 3) despite their similar ADG (Table 1). At the end of the experimental period, a greater ($P < 0.01$; Figure 3) number of LOWDENS were pubertal compared to HIDENS heifers (66.5 vs. 31.9 % pubertal heifers/total heifers; SEM = 5.8). Within heifers that reached puberty during the experiment, HIDENS were heavier ($P = 0.05$) and older ($P < 0.01$) at puberty attainment compared with LOWDENS heifers (328 vs. 363 d of age, SEM = 12; 319 vs. 372 kg of BW, SEM = 11; respectively).

Collectively, these results indicate that rearing heifers in high stocking density delayed their onset of puberty despite adequate age and BW development, likely due to treatment differences among physical activity and chronic stress parameters. Physical activity causes release of endogenous opiods, which modulate gonadotropin secretion and consequent onset of puberty, cyclicity, and fertility in cattle (Mahmoid et al., 1989). Accordingly, Lamb et al. (1979) reported that prepartum exercise regimens enhanced subsequent reproductive efficiency in dairy heifers without impacting BW change. Regarding stress and puberty attainment, chronic stress and the resultant increase in adrenocortical activity impairs gonadotrophin synthesis and release (Dobson et al., 2000) and reduces the sensitivity of the brain to estrogen (Hein and Allrich, 1992). Hence, the reduced physical activity and increased adrenocortical activity of HIDENS heifers, as evidenced by treatment differences on steps/week, blood mRNA expression of HSP, and hair cortisol concentrations, likely contributed to their delayed puberty attainment compared with LOWDENS cohorts.

IMPLICATIONS

In conclusion, rearing replacement beef heifers in drylots with high stocking density negatively impacted stress-related and physiological responses, and delayed puberty attainment compared with rearing heif-

ers in pastures with low stocking density. Moreover, these outcomes were independent of heifer nutritional status and growth rate, but were associated with reduced physical activity and increased chronic stress caused by high stocking density. Therefore, stocking density should be considered in heifer development programs to optimize reproductive and overall efficiency of cow-calf operations.

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Effects of one or two prostaglandin $F_{2\alpha}$ injections on progesterone concentrations and luteolysis in suckling beef cows subjected to a five-day controlled internal drug release–Co-Synch protocol[1]

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ABSTRACT: The objective of this study was to examine the effects of 1 or 2 doses of conventional $PGF_{2\alpha}$ (PG), or 1 high concentration, long-lasting PG (HighCon) on progesterone (P_4) concentration profiles, and luteal function in suckling beef cows subjected to a 5-d controlled internal drug release (CIDR)-Cosynch protocol. On d 0, thirty-one cows received GnRH (100 µg; i.m.), and a CIDR was inserted. On d 5, the CIDR was removed and cows were assigned randomly to receive 1 dose of PG (1PG; 25 mg; n = 11), 2 doses (25 mg/dose) of PG 12 h apart (2PG; n = 10), or 1 high concentration, long-lasting PG (HighCon; 25 mg; n = 10). On d 5, one h after CIDR removal, serial blood samples were collected every 12 h from d 5 to 8 to measure P_4 concentrations. On d 8, all cows received a second GnRH injection and were inseminated. Ovaries were examined by ultrasonography on d 0, 5 and 8, and ovarian structures were recorded. A repeated measures, generalized linear mixed model ANOVA was used to determine differences in P_4 concentrations. The model included treatment, the repeated factor time, and time by treatment interaction as fixed effects, and cow within treatment was the random effect. The P_4 data were assumed to follow a lognormal distribution. On d 5, all cows had a CL, and P_4 concentrations were similar between groups ($P = 0.90$) prior to treatment administration. As expected, P_4 concentrations decreased over time in all treatments ($P < 0.01$). There was no treatment ($P > 0.1$), or treatment by time interaction ($P > 0.1$) on P_4 concentrations. By 60 h and 72 h post-treatment, the average P_4 concentrations were 0.1 ng/mL and 0.1 ng/mL for 1PG, 0.06 ng/mL and 0.05 ng/mL for 2PG, and 0.08 ng/mL and 0.07 ng/mL for HighCon, respectively. These results demonstrate that 1PG, 2PG (12 h apart), or 1 HighCon in a 5-d CIDR-Cosynch protocol for suckling beef cows will result in similar P_4 concentrations by the time of AI. Thus, 1 injection of PG is as effective as 2PG (12 h apart) in causing complete luteolysis by the time of AI as P_4 concentrations were similar.

Key words: beef cow, five-day controlled internal drug release–Co-Synch, luteolysis, progesterone, prostaglandin $F_{2\alpha}$

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INTRODUCTION

Estrous synchronization protocols that result in greater synchronized ovulation and pregnancy per AI (**P/AI**) will facilitate the use of AI, and may increase its adoption among beef producers. The use of Cosynch protocols have allowed for synchronization of ovulation and timed AI (**TAI**), as well as reduced number of handlings when compared to Ovsynch (Geary and Whittier, 1998). The addition of controlled internal drug release (**CIDR**) inserts between the initial GnRH and $PGF_{2\alpha}$ (**PG**) improve synchronization, and P/AI by preventing premature estrus and ovulation before PG (Xu and Burton, 2000; Larson et al., 2006).

Reducing the duration of CIDR treatment to the induction of luteolysis from 7 to 5 d in the CIDR-Cosynch, has shown to increase P/AI in beef cows (Bridges et al., 2008). However, it was concluded that in this protocol, 2 injections of PG (**2PG**, 7 to 24 h apart) on d 5 are required to reliably induce luteolysis by the time of AI (Bridges et al., 2008).

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Using a 5-d CIDR-Cosynch, some studies have shown improved P/AI in beef cows receiving 2PG compared with 1 PG (**1PG**) (Kasimanickam et al., 2009; Whittier et al., 2010). It was hypothesized that improved P/AI may be attributable to efficacy of 2PG in causing complete luteolysis and lower progesterone (**P_4**) by the time of AI compared with 1PG. Interestingly, P_4 concentrations following PG, and ovarian status were not assessed in any of these published studies to compare the effect of 1PG vs. 2PG on P_4 and ovarian status by AI. Moreover, the primary concerns with 2PG injections in a 5-d CIDR-Cosynch protocol for beef cows is the increase number of animal handling, and drug and labor costs. Given the lack of evidence on the effects of 1PG vs. 2PG on luteal function and P_4 in a 5-d CIDR-Cosynch protocol in beef cows, the objectives of this study were to examine the effects of 1PG, 2PG (12 h apart), and 1 long-lasting, (**HighCon**) PG on luteolysis and P_4 profiles in suckling beef cows subjected to a 5-d CIDR-Cosynch protocol.

MATERIALS AND METHODS

All procedures and protocols were in compliance with the University of Idaho, Animal Care and Use Committee. Thirty-one suckling Charolais beef cows from the University of Idaho (Moscow, Idaho) research facility were used for this study. All cows were on average 58 days postpartum.

Experimental Design and Treatments

Seven days before synchronization, jugular blood samples were collected from all cows (Fig. 1). On d 0 (initiation of synchronization), all cows received GnRH (100 μg, i.m.; Factrel; Fort Dodge Animal Health, Fort Dodge, IA), and a blood sample was obtained. Simultaneously, a CIDR (1.38 g P_4; Eazi-Breed CIDR, Zoetis, Florham Park, NJ) was inserted (Fig. 1), and all cows were subjected to transrectal ultrasonography (Aloka SSD-500 V; Aloka, Tokyo, Japan), and categorized by presence or absence of a corpus luteum (**CL**). Five d later CIDR inserts were removed, and cows were stratified by days postpartum, and presence or absence of a CL on d 0 then assigned randomly to 1 of 3 treatments: 1 PG (1PG; n = 11; 25 mg, i.m.; Lutalyse, Zoetis, Florham Park, NJ), 2 PG 12 h apart (2PG; n = 10; 50 mg total, i.m.; Lutalyse, Zoetis, Florham Park, NJ), or 1 high concentration, long-lasting PG (HighCon; n = 10; 25 mg, i.m.; Lutalyse HighCon, Zoetis, Florham Park, NJ) (Fig. 1). Before treatment, blood samples were taken and ultrasonography was conducted to confirm the presence of luteal tissue. Following treatment, blood samples were collected every 12 h from d 5 to 8 to measure P_4 concentrations (Fig. 1). Between d 5 and 8, cows were monitored every 4 to 6 h for estrus behavior via visual observation and removal of tail chalk painting. Regardless of estrus expression, all cows received a second GnRH (100 μg) and inseminated by 1 inseminator on d 8 (Fig. 1). Additionally, on d 8 ovarian structures were examined recorded by ultrasongraphy. Blood samples were taken 11 d (d 19) post-AI for P_4 concentrations, and pregnancy status was confirmed 32 d post-AI using pregnancy specific protein B analysis (BioPryn; BioTracking, Inc., Moscow, Idaho; Fig. 1).

Ovarian Examination

On d 0, 5, and 8 transrectal ultrasonography (Aloka SSD-500 V; Aloka, Tokyo, Japan) was conducted to assess ovarian structures. Cows were stratified into treatments on d 5 based on the presence or absence of a CL on d 0. Additionally, cows were categorized as developing a new CL, or possessing an old CL at the time of treatment. The presence of a dominant follicle (≥ 10 mm diameter) on d 0, and the presence of a CL on d 5 in the same location identified cows that ovulated to the initial GnRH and formed a new CL. Cows presenting a CL on d 0 and d 5 in the same location were classified as possessing an old CL at the time of treatment. On d 8, the presence of a dominant follicle (≥ 10 mm diameter) indicated potential ovulatory follicle at the time of the second GnRH and AI.

Blood Samples and Progesterone Quantification

Blood samples were collected via jugular venipuncture using an 18 gauge, 1 ½" single use blood collection needle. Blood samples were collected using a 10 mL vaccumed tube (Covidien LLC, Mansfield, MA). All samples were placed on ice and stored at 4°C for 18 to 24 h. Samples were then centrifuged for 20 min at 2,400 × g and 4°C. Serum was harvested and stored at -20°C until assayed for P_4 concentrations. Progesterone concentrations were analyzed using a double antibody, RIA (MP Biomedicals, Costa Mesa, CA) under equilibrium conditions. The standard curve ranged from 0.05 to 25 ng/mL, and all samples and standards were run in duplicates, with an intra- and inter-assay, coefficient of variance of 3.6% and 6.7%, respectively.

Statistical Analysis

In SAS 9.4 (SAS Inst. Inc., Cary, NC), the GLM procedure was used to analyze the descriptive statistics of days postpartum, and BW. Based on the absence or presence of a CL on d 0 and d 5, cows were classified

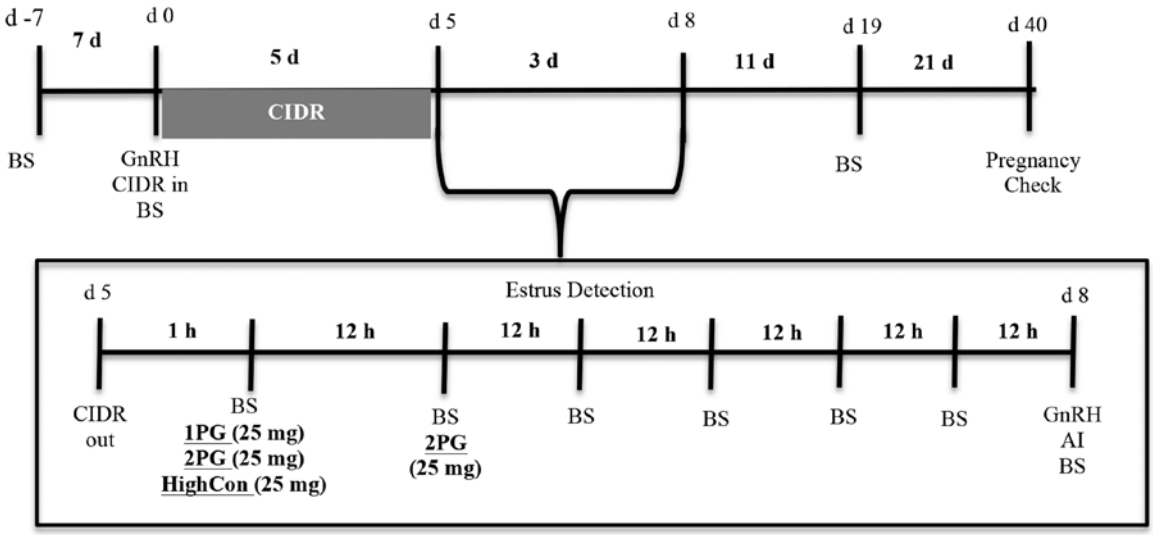

Figure 1. Schematic of experimental design to examine the effect of 1 $PGF_{2\alpha}$ (1PG; n = 11; 25 mg, i.m), two (2PG; n = 10; 50 mg total, i.m.) 12 h apart, or 1 high concentration, long-lasting $PGF_{2\alpha}$ (HighCon; n = 10; 25 mg, i.m.) on progesterone concentrations in suckling Charolais beef cows subjected to a 5-d controlled internal drug release (CIDR)-Cosynch protocol. On d 0, 5, and 8, ovarian structures were recorded for all cows via transrectal ultrasonography (ULT). One h after CIDR removal, treatments were administered, and jugular blood samples (BS) were taken every 12 h until d 8, and additionally on d 11. Ultrasonography on d 8 was conducted to evaluate the effects of luteolysis between treatments. All BS were analyzed for P_4 concentrations to examine the differences in P_4 profiles following treatments.

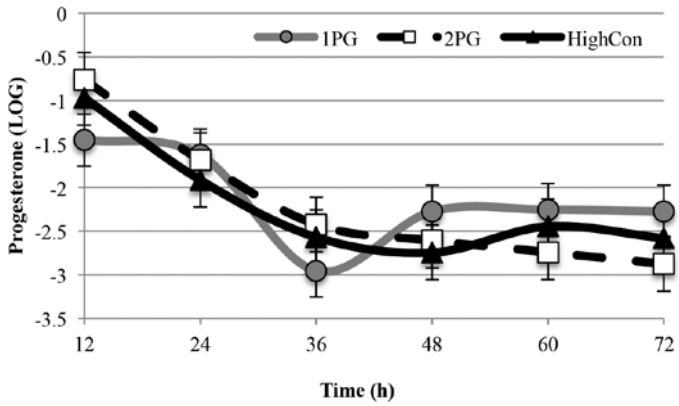

Figure 2. Progesterone (P_4) concentrations 12 h after controlled internal drug release (CIDR) removal and treatment (d 5) of either 1 $PGF_{2\alpha}$ (1PG; n = 11; 25 mg, i.m.; Lutalyse, Zoetis, Florham Park, NJ), 2 (2PG; n = 10; 50 mg total, i.m.; Lutalyse, Zoetis, Florham Park, NJ) 12 h apart, or one high concentration, long-lasting PG (HighCon; n = 10; 25 mg, i.m.) in suckling Charolais beef cows subjected to a 5-d CIDR-Cosynch protocol for first AI.

as developing a new CL (absence then presence) or having an existing CL (presence and presence) at the time of treatment (d 5). The GLM procedure was then used to determine differences on d 8 P_4 concentrations based on CL status (old vs. new) at the time of treatment, and the model included treatment, CL status (old vs. new), and treatment by CL status interaction.

A repeated measures, generalized linear mixed model ANOVA was used to determine differences in P_4 concentrations. The model included treatment, the repeated factor time, and time by treatment interaction as fixed effects. The random effect was cow within treatment, and P_4 concentrations prior to treatment (d 5) was used as a covariate in the model. The P_4 data were assumed to follow a lognormal distribution. All statistical inferences were made based on the lognormal data, and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

There were no differences in P_4 concentrations between groups before treatments ($P = 0.9$). Mean P_4 concentrations on d 5 were 1.65 ng/mL for 1PG, 1.60 ng/mL for 2PG, and 1.92 ng/mL for HighCon. As expected, P_4 concentrations decreased over time ($P < 0.01$) between d 5 and 8, regardless of treatment (Fig. 2). There were no treatment ($P = 0.7$) or treatment by time ($P > 0.1$) interactions on P_4 concentrations between treatment groups (Fig. 2). Progesterone concentrations did not

TABLE 1. Mean ± SE for BW and days postpartum in suckling Charolais beef cows given 1 $PGF_{2\alpha}$ (1PG; n = 11; 25 mg, i.m.), 2 (2PG; n = 10; 50 mg total, i.m.) 12 h apart, or 1 high concentration, long-lasting PG (HighCon; n = 10; 25 mg, i.m.) following controlled internal drug release (CIDR) removal in a 5-d CIDR-Cosynch protocol for first AI

Treatment	BW (kg)	Days postpartum (d)
1PG (n = 11)	688.78 ± 11.44	58 ± 1
2PG (n = 10)	668.94 ± 12.06	57 ± 1
HighCon (n = 10)	688.70 ± 14.77	58 ± 1

differ between treatment groups by 60 h ($P = 0.53$) and 72 h ($P = 0.38$) post-treatment. Sixty h post-treatment, P_4 concentrations were 0.10 ng/mL, 0.06 ng/mL and 0.08 ng/mL, and by 72 h post-treatment, P_4 concentrations were 0.10 ng/mL, 0.05 ng/mL and 0.07 ng/mL for 1PG, 2PG, and HighCon, respectively.

There were no differences in average days postpartum ($P = 0.7$) or BW ($P = 0.4$) between treatment groups (Table 1). Ovarian structures mapped and recorded on d 0 and 5 indicated that the proportion of cows that developed a new CL due to ovulation by the initial GnRH was 50% regardless of treatment. These results are consistent with other studies, which have indicated that approximately 50% of cows will ovulate to the initial GnRH when synchronization is initiated without presynchronization and during random stages of the estrous cycle (Vasconcelos et al., 1999; Stevenson, 2016). There was no treatment ($P = 0.26$), CL status (new vs. old; $P = 0.92$), or treatment by CL status interactions ($P = 0.90$) on P_4 concentrations by d 8 and time of AI. All cows were cyclic and had a CL prior to administration of treatment on d 5. On d 8, 9 cows (4 in 1PG, 3 in 2PG, and 2 in HighCon) had remnants of luteal tissue based on ultrasonography.

Previous researchers hypothesized that P/AI in CIDR-Cosynch protocols would be increased if the interval from the initial GnRH to PG and CIDR withdrawal was shortened from 7 to 5 days (Bridges et al., 2008; Kasimanickam et al., 2009; Whittier et al., 2010). However, the success of synchronization relies on the ability of PG to regress a GnRH-induced, newly formed CL, and reduce P_4 concentrations by the time of AI. These investigators assert that if ovulation to the initial GnRH (at CIDR insertion) occurs, the GnRH-induced CL will be immature and unable to respond to a single injection of PG with complete luteolysis in the 5-d CIDR-Cosynch. Further, this incomplete luteolysis is believed to result in a suboptimal P_4 concentration (at the time AI) and lower fertility. Thus, 2PG injections are warranted. These researchers postulated that the apparent improved P/AI in cows that received 2PG injections is attributable to the effectiveness of a double-injection scheme in causing complete luteolysis and optimal P_4 concentrations at the time of AI. Nevertheless, the association between complete and incomplete luteolysis and fertility was not tested in the above studies (Bridges et al., 2008; Kasimanickam et al., 2009; Whittier et al., 2010) as blood P_4 concentrations after PG were not measured and ovarian structures were not examined.

The current study provides the first evidence on P_4 concentration profiles, and ovarian dynamics following 1PG, 2PG or HighCon in suckling beef cows subjected to a 5-d CIDR-Cosynch protocol. The results showed that the administration one or two doses of $PGF_{2\alpha}$ do not differ in the rate of P_4 decline, or serum P_4 concentrations by the time of AI. Interestingly, one injection, of HighCon, is as effective in causing complete luteolysis and lowering P_4 concentrations as 2PG. Thus the apparent improved P/AI in cows received 2PG in some studies may not be due to reducing P_4 by the time of AI and perhaps other underlying factors are involved.

In the current study P_4 concentrations at the time of AI were similar between all treatment groups. These findings are similar to those observed by Ahmadzadeh et al. (2011). In that study, the researchers investigated the effects of 1 PG (25 mg), 1 ½ PG (37.5 mg), or ½ dose of PG (12.5 mg) given 7 h apart following CIDR removal in a 5-d CIDR-Cosynch protocol in beef cows (Ahmadzadeh et al., 2011). Progesterone concentrations were measured at CIDR insertion and at the time of AI. Ahmadzadeh et al. (2011) found no difference in P_4 concentrations regardless of treatment, and all treatments had < 1 ng/mL by the time of AI.

It has been shown that P/AI is greater when P_4 concentrations are high at the time of PG and low (< 0.5 ng/mL) by the time of AI (Stevenson, 2016). Based on these results, 1PG and 1 injection of HighCon are as effective in causing complete luteolysis and reducing P_4 concentrations to < 0.5 ng/mL by the time of AI as 2PG. However, further research is needed in order to confirm the relationship of P_4 concentrations and complete luteolysis on P/AI in beef cows synchronized with a 5-d CIDR-Cosynch protocol.

IMPLICATIONS

This study provides evidence on progesterone concentrations profiles following 1 or 2 prostaglandin, or 1 high concentration, long-lasting prostaglandin in beef cows subjected to a 5-d controlled internal drug release Cosynch protocol. The investigation of the use of 1 or 2 doses of prostaglandin injections in the

5-d Cosynch protocol was developed to gain further knowledge regarding luteolysis and progesterone concentrations, and weigh the potential influence on pregnancy against additional cattle handling and expenses associated with a second prostaglandin. Considering progesterone concentrations and its relationship to fertility, the administration of 1 prostaglandin is more practical for synchronizing beef cows as it reduces the number of animal handling, labor, and drug costs. Future research is needed in order to compare the effects of progesterone profiles on pregnancy in order to further support the use of 1 prostaglandin injection in a 5-d Cosynch protocol for beef cows.

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Effects of rumen-protected EFA supplementation to late-gestating beef cows on performance and physiological responses of the offspring

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ABSTRACT: This experiment compared performance and physiological responses of the offspring from cows supplemented with a rumen-protected EFA or SFA + MUFA source during late gestation. Ninety-six multiparous, non-lactating, pregnant Angus × Hereford cows were stratified by BW and BCS, and divided into 24 groups of 4 cows/group at the end of their 2nd trimester of gestation (d -7). All cows became pregnant during the same estrus-synchronization + AI protocol, with semen from a single sire. Groups were randomly assigned to receive (as-fed basis) 454 g/cow daily of soybean meal in addition to 1) 200 g/cow daily of rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids or 2) 200 g/cow daily of rumen-protected SFA + MUFA mix based on palmitic and oleic acids (CON). Groups were maintained in 2 pastures (6 groups of each treatment/pasture), and received daily 10.9 kg/cow (as-fed basis) of grass-alfalfa hay. Groups were segregated and offered treatments 3 times/week from d 0 until calving. Cow BW and BCS were recorded, and blood samples were collected on d -7 and within 12 h after calving. Calf BW was also recorded within 12 h of calving. Calves were weaned on d 280 of the experiment, preconditioned for 45 d (d 280 to 325), transferred to a growing lot on d 325, and moved to a finishing lot on d 445 where they remained until slaughter. At calving, EFA-supplemented cows had greater ($P < 0.01$) proportion (as % of total plasma fatty acids) of PUFA including linoleic, linolenic, arachidonic, docosapentaenoic, and docosahexaenoic acids. At weaning, calves from CON-supplemented cows were older ($P = 0.03$), although no treatment differences were detected ($P = 0.82$) for calf weaning BW. During both growing and finishing phases, ADG was greater ($P \leq 0.06$) in calves from EFA-supplemented cows. Upon slaughter, HCW and marbling were also greater ($P \leq 0.05$) in calves from EFA-supplemented cows. Collectively, these results suggest that supplementing EFA to late-gestating beef cows stimulated programming effects on postnatal offspring growth and carcass quality. Thus, supplementing late-gestating beef cows with a rumen-protected EFA mix appears to optimize offspring productivity in beef production systems.

Key words: beef cows, EFA, offspring, pregnancy, supplementation
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INTRODUCTION

Maternal nutrition is a major extrinsic factor programming nutrient partitioning and consequent development of fetal organ systems associated with health, production, and reproduction (Funston et al., 2010). Hence, nutritional management of late-gestating beef cows has been shown to directly impact performance of the subsequent offspring via fetal programming effects (Marques et al., 2016a). However, the majority of research conducted to date within this subject focused on energy and CP nutrition, and little is known about the potential impacts of supplementing EFA to gestating cows on offspring productivity.

In humans and livestock species, ω-3 and ω-6 fatty acids (**FA**) are considered essential by playing critical roles in several body functions but cannot be synthesized by the body; hence, EFA must be

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consumed through the diet (Hess et al., 2008). During gestation, dietary EFA become available in the circulation and are transferred to the fetus via the placenta (Garcia et al., 2014). In humans, supplementing pregnant women with EFA is considered critical for optimal fetal and early-life child development, including growth, nervous, and immune responses (Greenberg et al., 2008). Accordingly, research with swine reported that supplementing pregnant sows with EFA benefited piglet vitality, as well as pre- and post-weaning growth (Tanghe and De Smet, 2013).

Based on these research, we hypothesized that supplementing EFA to late-gestating beef cows will increase postnatal offspring productivity. Nevertheless, EFA should be supplement to cattle and other ruminant livestock as rumen-inert sources to prevent extensive ruminal biohydrogenation (Hess et al., 2008). Hence, this experiment evaluated the effects of rumen-protected EFA supplementation to beef cows during the last trimester of gestation on performance and physiological responses of the offspring.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns station). The animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4758).

Cow-calf management and dietary treatments.

Ninety-six multiparous, non-lactating, pregnant Angus × Hereford cows (BW = 586 ± 4 kg, age = 7.5 ± 0.2 yr, BCS = 5.01 ± 0.03 according to Wagner et al., 1988) were assigned to this experiment at the end of their 2nd trimester of gestation. Cows were pregnant to fixed-time AI using semen from a single Angus sire (d 195 of gestation on d 0).

Prior to the beginning of the experiment (d -7), cows were stratified by BW and BCS, and divided into 24 groups of 4 cows/group. Groups were then randomly assigned to receive (as-fed basis) 454 g of soybean meal per cow daily in addition to 1) 200 g/cow daily of rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (100 g of Prequel + 100 g of Strata; Virtus Nutrition LLC., Corcoran, CA) or 2) 200 g/cow daily of rumen-protected SFA + MUFA mix based on palmitic and oleic acids (**CON**; 200 g of EnerGII; Virtus Nutrition). Supplement treatments were iso-nitrogenous, iso-lipidic, and iso-caloric (Table 1). Groups were maintained in 1 of 2 meadow foxtail pastures (12 groups/pasture, being 6 groups/treatment in each pasture) beginning on d -7. Grass-alfalfa hay was provided daily at 10.9 kg/cow (as-fed basis), and cows had ad libitum access to water and a commercial mineral + vitamin mix.

TABLE 1. Ingredient composition and nutrient profile of diets containing a rumen-protected SFA + MUFA mix (CON) based on palmitic and oleic acids, or a rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids

Item	CON	EFA
Ingredients, kg/day (as-fed basis)		
Grass-alfalfa hay	10.9	10.9
Soybean meal	0.454	0.454
EnerGII [1]	0.200	-
Prequel [1]	-	0.100
Strata [1]	-	0.100
Nutrient profile [2] (DM basis)		
DM	93.5	93.5
TDN, %	61	61
NEm, Mcal/kg	1.29	1.28
CP, %	10.2	10.2
Fat, %	3.52	3.49
Palmitic acid (16:0), %	0.88	0.49
Oleic acid (18:1), %	0.91	0.61
Linoleic acid (18:2), %	0.44	0.69
Linolenic acid (18:3), %	0.92	0.97
Eicosapentaenoic acid (20:5n-3), %	0.00	0.13
Docosahexaenoic acid (22:6n-3), %	0.00	0.11

[1]Ca salts by Virtus Nutrition LLC (Corcoran, CA).

[2]Values obtained via wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

From d 0 of the experiment until calving, cows were gathered 3 times weekly and groups were sorted into 1 of 12 drylot pens. Groups were offered treatments individually (6.08 kg of supplement treatment/feeding per group; as-fed basis) Diets (hay + treatments) were formulated to meet or exceed nutrient requirements for energy, protein, minerals, and vitamins of late-gestating beef cows (NRC, 2000). Immediately after calving, cow-calf pairs were removed from their pasture, and assigned to the general management of the research herd that did not include rumen-inert EFA or SFA + MUFA supplementation (Marques et al., 2016a).

Calf management

Preconditioning (d 280 to 325). Calves were weaned on d 280 of the experiment and transferred to a 6-ha meadow foxtail pasture for a 45-d preconditioning period as a single group (Marques et al., 2016b). During preconditioning, calves received mixed alfalfa-grass hay (12% CP, 57% TDN; DM basis), water,

and commercial mineral and vitamin mix for ad libitum consumption.

Growing (d 325 to 445) and finishing (d 445 until slaughter). On d 325, all calves were loaded into a commercial livestock trailer and transported for 480 km to the growing lot (Top Cut; Echo, OR), where they remained for 120 d and managed as a single group. On d 445, calves were moved to an adjacent finishing lot (Beef Northwest; Boardman, OR), where they continued to be managed as a single group until slaughter at a commercial packing facility (Tyson Fresh Meats Inc., Pasco, WA). Growing and finishing diets, which did not contain rumen-protected EFA or SFA + MUFA, were fed ad libitum as described in Marques et al. (2016b).

Sampling

Feedstuffs. Two samples of all dietary ingredients fed to late-gestating cows were collected before the beginning of the experiment and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Feed samples were also analyzed for FA profile.

Cows and newborn calves. Individual cow BW and BCS were recorded and a blood sample was collected prior to the beginning of the experiment (d -7). Within 12 h after calving, cow BW, cow BCS, calf birth BW and calf gender were recorded, and blood was collected from each cow.

Preconditioning. Cow BW and BCS were recorded at weaning (d 280). Calf BW was recorded and blood samples were collected on d 280, 282, 285 and 288 of the experiment. During the 45 d of preconditioning, calves were observed daily for bovine respiratory disease (**BRD**) signs and treated when signs were observed.

Growing and finishing. Calf BW was recorded upon arrival at the growing lot (d 325) and the finishing lot (d 425). Calves were observed daily for BRD signs and received medication according to the management criteria of the growing and finishing yards. At the commercial packing plant, carcass traits were collected upon slaughter.

Blood analysis

All blood samples were collected via jugular venipuncture, centrifuged at 2,500 × *g* for 30 min for plasma collection, and stored at -80°C on the same day of collection. Samples from cows (d -7 and after calving) were analyzed for FA profile (Garcia et al., 2014). Samples collected from calves from d 280 to 288 were analyzed for haptoglobin and cortisol concentrations (Marques et al., 2016b).

Statistical analysis

All cow and calf variables were analyzed with group as the experimental unit, and group(treatment × pasture), cow(group), and pasture as random variables. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). All data was analyzed using gestation days receiving treatment as an independent covariate, and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Model statements for cow-related responses included the effects of treatment. Analysis of cow plasma FA profile at calving also included results from d -7 as independent covariate. Model statements for calf-related responses analysis included the effects of treatment, calf gender as independent covariate, as well as day and treatment × day interaction for plasma haptoglobin and cortisol analyses. Finishing lot and carcass variables analyses also included days on feed as an independent covariate. The specified term used in the repeated statement for plasma haptoglobin and cortisol was day, the subject was cow(group), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the lowest Akaike information criterion. Results are reported as covariately-adjusted least square means, and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

RESULTS AND DISCUSSION

Nutrient composition and profile of diets offered to EFA- and CON-supplemented cows are described in Table 1. Both diets were formulated to represent a typical forage-based diet with limited fat content, and provided adequate amounts of energy and CP based on the requirements of pregnant cows during last trimester of gestation (NRC, 2000). It is important to note that both diets included the same amount of rumen-protected fat, which were based on Ca salts but differed in FA profile. The CON treatment was included to serve as an iso-lipidic, iso-caloric, and iso-nitrogenous control treatment to EFA. Hence, results from this experiment should not be associated with differences in total nutrient or FA intake, but with potential fetal programming effects of supplemental ω-3 and ω-6 EFA.

Cow parameters

As designed, initial cow BW and BCS (d -7) were similar ($P \geq 0.75$) among treatments (Table 2). No treatment effects were detected ($P \geq 0.20$) for any of the subsequent BW and BCS parameters evalu-

ated (Table 2). These outcomes were expected given EFA- and CON-supplemented cows consumed similar amounts of energy and CP during late gestation, and were managed as a single group from calving until weaning. Cows assigned to the EFA and CON supplements had similar ($P \geq 0.11$) proportion (as % of total plasma FA) of all plasma FA on d -7 (data not shown), indicating similar FA profile before treatment administration. At calving, EFA-supplemented cows had greater ($P < 0.01$) proportion of plasma vaccenic, linoleic, linolenic, arachidonic, docosapentaenoic, and docosahexaenoic acids, as well as total PUFA, ω-3, and ω-6 compared with CON-supplemented cows (Table 3). Cows supplemented CON had greater ($P < 0.01$) proportion of plasma palmitic, stearic, oleic, eicosapentaenoic, and lignoceric acids, as well as total SFA and MUFA compared with EFA-supplemented cows at calving (Table 3). Overall, these results are in accordance with the FA content and intake of treatments, given that plasma profile reflects intake and intestinal FA flow (Hess et al., 2008).

Calf birth and weaning parameters

No treatment effects were detected ($P \geq 0.16$; Table 4) for any of the calving and weaning parameters evaluated. Others have also reported similar birth and weaning BW in calves from cows supplemented or not with EFA during gestation (Hess et al., 2008). Collectively, calving and weaning results indicate that supplementing late-gestating beef cows with EFA did not impact offspring growth during gestation, as well as growth from birth to weaning compared with CON-supplemented cohorts.

Calf preconditioning parameters

No treatment effects were detected ($P = 0.20$) herein for plasma cortisol, which increased (day effect; $P < 0.01$) for both treatments upon weaning (28.5, 31.7, 32.7, and 28.4 ng/mL on d 280, 282, 285, and 288, respectively; SEM = 1.2). A treatment × day interaction was detected ($P = 0.05$) for plasma haptoglobin concentrations, which also increased for both treatments upon weaning (day effect; $P < 0.01$) but was greater ($P = 0.05$) in calves from CON-supplemented cows on d 282 (1.70 vs. 1.51 mg/mL, respectively; SEM = 0.07). These outcomes suggest that EFA supplementation to late-gestating cows did not impact the steroidogenesis required to cope with the stress of weaning procedures in the offspring, but altered the resultant plasma haptoglobin protein response (Araujo et al., 2010). During the 45-d preconditioning period, no treatment effects were detected ($P \geq 0.23$) for incidence of calves that required treatment for BRD, calf mortality, ADG, and BW at the end of preconditioning period (Table 4). Hence, calf preconditioning responses were not impacted by treatments despite differences detected for plasma haptoglobin concentration, which has been negatively associated with performance and health parameters in weaned cattle (Araujo et al., 2010).

TABLE 2. Performance of beef cows receiving diets supplemented with a rumen-protected SFA + MUFA mix (CON) based on palmitic and oleic acids (n = 12), or a rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (n = 12) during the last trimester of gestation[1]

Item	CON	EFA	SE	*P* =
Days receiving diets, d	87.0	88.8	0.6	0.02
Initial (d -7)	584	589	15	0.75
Calving	616	614	11	0.90
BW change	31	25	4	0.20
Weaning (d 280)	575	567	10	0.63
BW change	-41	-45	12	0.43
BCS				
Initial (d -7)	5.01	5.02	0.05	0.89
Calving	5.41	5.46	0.06	0.59
BCS change	0.41	0.42	0.07	0.88
Weaning (d 280)	5.08	5.00	0.08	0.38
BCS change	-0.33	-0.47	0.10	0.26

[1]CON = cows received (as-fed basis) 200 g/cow daily of rumen-protected fatty acid mix based on palmitic and oleic acids (EnerGII; Virtus Nutrition, LLC, Corcoran, CA); EFA = cows received (as-fed basis) 200 g/cow daily of rumen-protected essential fatty acids mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (100 g Prequel + 100 g of Strata; Virtus Nutrition). Treatments were provided from d 0 until calving.

Calf feedlot and carcass parameters

During the growing lot phase, no treatment effects were detected ($P \geq 0.52$) for initial growing lot BW and proportion of calves treated for BRD symptoms. Calves from EFA-supplemented cows had greater ($P = 0.05$) ADG and tended to be heavier ($P = 0.09$) at the end of the growing lot phase compared with calves from CON-supplemented cows (Table 5). During the finishing lot, the proportion of animals treated for BRD symptoms, days on feed (Table 5), % of calves slaughtered, and % of male calves slaughtered (data not shown) were also similar ($P \geq 0.16$) among treatments. Calves from EFA-supplemented cows tended to have greater ($P = 0.06$) ADG and were heavier ($P = 0.05$) at the end of the finishing phase compared with calves from CON-supplemented cows (Table 5). Upon slaughter, HCW and marbling were greater ($P \leq 0.05$) whereas LM area and % Choice carcasses tended to be greater ($P \leq 0.10$) in calves from EFA-supplemented vs. CON-supplemented cows (Table 5). No treatment differences were detected ($P \geq 0.38$) for the remaining carcass traits evaluated (Table 5).

TABLE 3. Plasma fatty acid profile (g/100 g of plasma fatty acids) at calving of beef cows receiving diets supplemented with a rumen-protected SFA + MUFA mix (CON) based on palmitic and oleic acids (n = 12), or a rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (n = 12) during the last trimester of gestation[1]

Item	CON	EFA	SE	*P* =
Palmitic (16:0)	26.7	17.9	1.7	<0.01
Stearic (18:0)	25.8	18.7	2.4	<0.01
Oleic (18:1)	13.5	7.0	0.2	<0.01
Vaccenic (18:1 trans-11)	0.55	0.79	0.02	<0.01
Linoleic (18:2 n-6)	19.5	38.7	3.1	<0.01
Gamma-linolenic (18:3 n-6)	0.20	0.12	0.08	0.15
Linolenic (18:3 n-3)	2.01	3.73	0.59	<0.01
CLA (18:2 n-6 isomers)	0.08	0.11	0.02	0.14
Arachidonic (20:4 n-6)	0.55	2.08	0.19	<0.01
Eicosapentaenoic (20:5 n-3)	0.10	0.01	0.03	<0.01
Behenic (22:0)	0.60	0.40	0.17	0.10
Docosapentaenoic (22:5 n-3)	0.10	0.44	0.06	<0.01
Docosahexaenoic (22:6 n-3)	0.01	0.57	0.05	<0.01
Lignoceric (24:0)	0.07	0.04	0.01	<0.01
Total SFA	58.8	41.6	4.2	<0.01
Total MUFA	17.8	11.9	0.3	<0.01
Total PUFA	22.6	44.9	4.1	<0.01
Total ω-3	2.2	4.8	0.6	<0.01
Total ω-6	20.4	41.1	3.4	<0.01

[1]CON = cows received (as-fed basis) 200 g/cow daily of rumen-protected fatty acid mix based on palmitic and oleic acids (EnerGII; Virtus Nutrition, LLC, Corcoran, CA); EFA = cows received (as-fed basis) 200 g/cow daily of rumen-protected essential fatty acids mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (100 g Prequel + 100 g of Strata; Virtus Nutrition). Treatments were provided from d 0 until calving. Blood samples were collected from all cows (n = 48 per treatment) within 12 h after calving.

TABLE 4. Calving, weaning, and preconditioning outcomes from beef cows receiving diets supplemented with a rumen-protected SFA + MUFA mix (CON) based on palmitic and oleic acids (n = 12), or a rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (n = 12) during the last trimester of gestation[1]

Item	CON	EFA	SE	*P* =
Calving results				
% of male calves born	46.8	56.8	7.5	0.34
Calf birth BW, kg	40.9	41.7	0.6	0.44
Adjusted calf birth BW,[2] kg	41.3	42.0	0.6	0.42
Weaning results				
% of male calves weaned	46.8	56.8	7.5	0.34
Calf weaning BW, kg	241	242	3	0.82
205-d adjusted weaning BW,[2] kg	258	259	3	0.86
Preconditioning results				
Treated for BRD symptom, %	6.8	3.8	3.8	0.55
Calf mortality, %	0.0	2.2	1.6	0.36
Preconditioning ADG, kg/d	0.43	0.50	0.05	0.31
End of preconditioning BW, kg	261	265	3	0.29

[1]CON = cows received (as-fed basis) 200 g/cow daily of rumen-protected fatty acid mix based on palmitic and oleic acids (EnerGII; Virtus Nutrition, LLC, Corcoran, CA); EFA = cows received (as-fed basis) 200 g/cow daily of rumen-protected essential fatty acids mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (100 g Prequel + 100 g of Strata; Virtus Nutrition). Treatments were provided from d 0 until calving.

Maternal nutrition impacts fetal muscle development via hyperplasia and hypertrophy, resulting in permanent effects on postnatal growth and performance (Du et al., 2010). During late-gestation, however, only muscle hypertrophy and adipocyte development are significantly influenced in the fetus by maternal nutritional status, with direct consequences on life-long growth and i.m. fat deposition (Du et al., 2010). Corroborating the treatment differences reported herein for ADG, HCW, LM area, and carcass marbling, EFA have been shown to impact muscle and adipocyte function in developing tissues. Hiller et al. (2012) reported that ω-3 FA positively regulates the expression of genes associated with muscle development and function, but reduced expression of genes regulating lipogenesis and FA accumulation in the LM to favor metabolism of muscle cells. On the other hand, ω-6 FA has been shown to have adipogenic effects by increasing the expression of PPARγ in muscle tissues; a key promoter of adipocyte differentiation and marbling in cattle (Moriel et al., 2014). Hence, the improvement in feedlot growth and carcass quality in calves from EFA-supplemented cows should be attributed to the combination of supplemental ω-3 and ω-6, whereas the specific role of each EFA deserves further investigation. By providing these EFA during late gestation, it can be speculated that accumulation of these FA into fetal tissues were increased, enhancing development of muscle and adipose cells, which translated into increased carcass growth and marbling when offspring was provided high-energy anabolic feedlot diets (Harper and Pethick, 2004).

IMPLICATIONS

Supplementing forage-fed beef cows during late gestation with a rumen-protected EFA mix based on equivalent amounts of ω-3 and ω-6 FA did not impact cow performance during gestation, calving rate, or calf birth BW. At calving, proportion of plasma ω-3 and ω-6 FA were greater in EFA-supplemented vs. CON-supplemented cows. No major differences in offspring performance, health, and immune parameters from birth to weaning and subsequent 45-d preconditioning. However, after being exposed to a high-energy feedlot diet, HCW was 16 kg heavier and carcass marbling increased from small to modest when comparing calves from EFA vs. CON-supplemented cows. These

TABLE 5. Feedlot performance and carcass characteristics of feeder cattle from beef cows receiving diets supplemented with a rumen-protected SFA + MUFA mix (CON) based on palmitic and oleic acids (n = 12), or a rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (n = 12) during the last trimester of gestation[1]

Item	CON	EFA	SE	*P* =
Growing lot performance				
Initial growing lot BW, kg	248	250	3	0.68
Respiratory disease signs, %	38.3	31.8	7.1	0.52
Growing lot ADG, kg/d	1.12	1.22	0.03	0.05
Final growing lot BW, kg	383	397	6	0.09
Finishing lot performance				
Days on feed, d	127	126	1	0.34
Treated for BRD symptoms, %	2.2	2.2	2.2	0.94
Final finishing lot BW, kg	621	646	9	0.05
Finishing lot ADG, kg/d	1.87	1.98	0.04	0.06
Carcass characteristics				
HCW, kg	391	407	6	0.05
Backfat, cm	1.74	1.82	0.09	0.38
LM area, cm^2	89.6	92.3	1.2	0.10
KPH, %	2.15	2.13	0.07	0.85
Marbling	489	539	16	<0.01
Yield grade	3.50	3.56	0.11	0.63
Retail product, %	48.6	48.4	0.3	0.56
Choice, %	93.5	100.0	2.7	0.09

[1]CON = cows received (as-fed basis) 200 g/cow daily of rumen-protected fatty acid mix based on palmitic and oleic acids (EnerGII; Virtus Nutrition, LLC, Corcoran, CA); EFA = cows received (as-fed basis) 200 g/cow daily of rumen-protected essential fatty acids mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids (100 g Prequel + 100 g of Strata; Virtus Nutrition). Treatments were provided from d 0 until calving.

results are suggestive of programming effects on postnatal offspring growth and health resultant from EFA supplementation to late-gestating cows. Hence, supplementing gestating beef cows with a rumen-protected EFA mix based on eicosapentaenoic, docosahexaenoic, and linoleic acids might be a feasible alternative to optimize offspring productivity and carcass quality in beef production systems.

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Effects of intravenous lipopolysaccharide administration on feed intake, ruminal forage degradability and liquid parameters, and physiological responses in beef cattle

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ABSTRACT: This experiment compared DMI, ruminal forage degradability and liquid parameters, and physiological responses in beef cattle receiving or not a lipopolysaccharide (**LPS**) challenge. Eight ruminally cannulated Angus × Hereford steers (485 ± 16 kg of BW) were housed in individual pens on d -7, ranked by BW, and allocated to 1 of 2 treatments administered on d 0: 1) i.v. bolus dose (0.5 μg/kg of BW, diluted in 5 mL of 0.9% sterile saline) of bacterial LPS (*Escherichia coli* 0111:B4), or 2) 5 mL of 0.9% physiological saline (i.v.; **CON**). Steers had free-choice access to mixed alfalfa-grass hay, water, and a commercial vitamin + mineral mix (d -7 to 6). Hay DMI was evaluated daily (d -5 to 6). Immediately prior to treatment administration (h 0), polyester bags containing 4 g of ground dietary hay (DM basis) were immersed into the rumen of each steer and incubated for 0, 4, 8, 12, 24, 36, and 48 h for DM and NDF degradability evaluation. Steers were intra-ruminally pulse-dosed with 5 g of Co-EDTA immediately prior to treatment administration, and rumen fluid samples were collected at 0, 2, 4, 6, 8, 12, 16, and 24 h for ruminal liquid volume and dilution rate calculations. Blood was collected every 2 h from -2 to 8 h, every 4 h from 8 to 16 h, every 12 h from 24 to 72 h, and every 24 h from 96 to 144 h. Steers receiving LPS had less ($P \leq 0.03$) DMI on d 0 and 1 compared with CON. Steers receiving LPS had reduced ($P \leq 0.05$) rumen liquid volume and dilution rate, ruminal disappearance rate and effective degradability of DM and NDF compared with CON. Steers receiving LPS had ($P \leq 0.05$) greater plasma tumor necrosis factor alpha at 2 h, greater plasma haptoglobin from 24 to 72 h, greater plasma cortisol from 12 to 16 h, greater serum NEFA from 6 to 48 h, greater plasma insulin and glucose at 2 h, reduced plasma glucose from 4 to 12 h, greater plasma cholecystokinin at 16 h, and greater plasma leptin concentrations at 8, 12, 16, 36, and 48 h relative to treatment administration. Hence, LPS administration transiently reduced DMI in steers via physiological reactions that modulate gastrointestinal motility and satiety centers in the central nervous system, in addition to potential host-microbiome endocrine interactions that impaired ruminal hay DM and NDF degradability.

Key words: beef cattle, inflammation, lipopolysaccharide, physiology, rumen function
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INTRODUCTION

The innate immune response plays a vital role in the organism's defense against disease and trauma, and includes the synthesis of hepatic acute-phase proteins in response to pro-inflammatory cytokines (Carroll and Forsberg, 2007). The acute-phase protein response is also triggered by stressful management procedures such as weaning and road transport, and has been observed to be detrimental to beef cattle performance (Cooke, 2017). Circulating concentrations of acute-phase proteins were negatively associated with ADG in cattle (Qiu et al., 2007) due to decreased DMI (Araujo et al., 2010), although the exact mechanisms for this latter outcome are not fully understood.

Rodrigues et al. (2015a) reported a transient decrease in DMI of beef cattle vaccinated against respiratory pathogens, which is known to stimulate the acute-phase response for proper acquirement of protective immunity. Authors attributed these results to metabolic and inflammatory components

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that impact satiety and digestive tract function, such as cortisol, tumor necrosis factor alpha (**TNFα**), insulin and leptin (Allen et al., 2009), as well as physical regulators of DMI including ruminal digestibility, motility, and passage rate (Allen, 2000).

The impacts of inflammatory and acute-phase responses on ruminal digestive function, however, still require investigation (Rodrigues et al., 2015a). One strategy to elicit the bovine acute-phase protein response and investigate such parameters is via lipopolysaccharide (**LPS**) administration (Rodrigues et al., 2015b). Hence, we hypothesized that LPS administration to cattle impacts rumen function and metabolic regulators of voluntary feed intake, resulting in decreased DMI. Based on this hypothesis, the objective of this experiment was to compare DMI, ruminal forage degradability and liquid parameters, as well as physiological and inflammatory responses in beef cattle administered or not LPS.

MATERIALS AND METHODS

All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4891).

Eight halter-trained, ruminally cannulated Angus × Hereford steers were utilized (d -7 to 6). During the experiment, steers were housed in an enclosed barn in individual pens (3 × 5 m) with free-choice access to mixed alfalfa-grass hay, water, and a commercial vitamin + mineral mix. Steers were weighed daily from d -3 to d -1 of the experiment, and values were averaged as initial BW (485 ± 16 kg). On d 0, steers were ranked by BW and assigned to 1 of 2 treatments (h 0): 1) i.v. bolus dose of bacterial LPS (0.5 μg/kg of BW; *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO; diluted in 5 mL of 0.9% sterile saline), or 2) 5 mL of 0.9% sterile saline (i.v.; **CON**).

Hay Intake and Ruminal Forage Disappearance

Hay DMI was evaluated daily from d -5 to 6, by weighing and collecting samples of the offered and nonconsumed feed. All samples were dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay daily DMI was calculated as kg/steer or based on % of initial BW.

Immediately before treatments were administered, Dacron bags (Ankom Technology Corp.) containing 4 g (DM basis) of ground dietary hay were suspended into the ruminal ventral sac of each steer, and incubated in triplicates for 0, 2, 4, 6, 8, 12, 24, and 48 h relative to treatment administration (h 0). After ruminal incubation, bags were washed repeatedly with running water until the rinse water was colorless, and subsequently dried for 96 h at 50°C in a forced-air oven. Dried samples were weighed for residual DM determination, and triplicates were analyzed for NDF (Robertson and Van Soest, 1981).

Liquid Dilution Rate and Volume

Immediately before treatments were administered (h 0), each steer was intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-mL aqueous solution via a stainless-steel probe with a perforated tip. Ruminal fluid samples (approximately 100 mL) were collected by suction strainer immediately prior to and at 2, 4, 6, 8, 12, 16, and 24 h after treatment administration. Twenty mL of ruminal fluid was stored (-20°C) until analysis of Co concentration by atomic absorption using an air/acetylene flame. Ruminal liquid volume and liquid dilution rate were estimated by regressing the natural logarithm of Co concentration against sampling time.

Sampling and Analyses

Blood samples were collected every 2 h from -2 to 8 h, every 4 h from 8 to 16 h, every 12 h from 24 to 72 h, and every 24 h from 96 to 144 h relative to treatment administration (h 0). Samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing no additive or containing 158 USP units of freeze-dried sodium heparin for serum and plasma collection, respectively. After collection, all blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection.

Serum samples collected from -2 to 60 h relative to treatment administration were analyzed for NEFA concentrations. Plasma samples collected from -2 to 60 h relative to treatment administration were analyzed for concentrations of glucose, leptin, cholecystokinin, cortisol, and insulin. Plasma samples collected from -2 to 8 h relative to treatment administration were analyzed for TNFα concentrations, whereas all plasma samples were analyzed for haptoglobin concentrations.

Statistical analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and

Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Steer was considered the experimental unit and steer(treatment) used as a random variable. Kinetic parameters of forage DM and NDF disappearance were estimated using nonlinear regression procedures of SAS (SAS Inst.). The model statement used for ruminal forage disappearance, liquid dilution rate, and rumen liquid volume contained the effect of treatment. The model statements used for hay intake and all blood variables contained the effects of treatment, time (day for DMI or hour for blood variables), and the resultant interaction. In addition, values obtained prior to treatment application (average daily DMI from d -5 to -1, or averaged values from -2 and 0 h for blood variables) were included as independent covariate in each respective analysis. The specified term for the repeated statement was day for DMI or hour for blood variables, steer(treatment) as subject, and the covariance structure used was first-order autoregressive based on the Akaike information criterion. Results are reported as covariately-adjusted least square means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

Steers receiving LPS had less ($P \leq 0.03$) DMI, expressed as kg/d or % of BW, compared with CON steers on d 0 and 1 of the experimental period (Figure 1). Steers receiving LPS had reduced rumen liquid dilution rate and liquid volume ($P \leq 0.05$) compared with CON steers (Table 1). Steers receiving LPS also had reduced ($P \leq 0.05$) ruminal disappearance rate and effective degradability of DM and NDF compared with CON steers (Table 1). Accordingly, treatment × hour interactions were detected ($P \leq 0.02$) for DM and NDF disappearance, as % remaining of initial DM and NDF content, given that these variables were less ($P < 0.01$) in LPS vs. CON steers from 4 to 12 h relative to treatment administration (Figure 2).

Treatment effects detected for hay DMI support our hypothesis, and agree with previous research reporting a transient decrease in voluntary feed intake following LPS administration (Steiger et al., 1999). Administration of LPS decreases ruminal contractions and impairs rumen motility (Lohuis et al., 1988). Decreased ruminal motility is known to decrease ruminal passage rate of liquids and solids, which directly regulates feed intake (Allen, 2000). Corroborating our results, Waggoner et al. (2009) reported that LPS administration decreased DMI and ruminal passage rate of liquid and solids in beef steers. Research investigating heat stress also observed a decrease in DMI and passage rate; however, DM and NDF digestibility were increased in heat-stressed dairy cattle (Bernabucci et al., 1999). In fact, reduced DMI, ruminal motility, and feed passage rate yields more time for ruminal feed digestion and increased ruminal volume (Farooq et al., 2010). In the present experiment, however, ruminal degradability of hay DM and NDF were

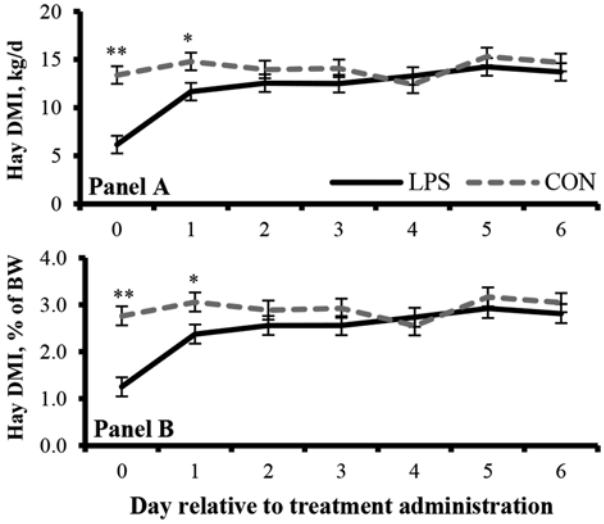

Figure 1. Hay DMI as kg/d (Panel A) or % of BW (Panel B) of steers receiving: 1) LPS (n = 4) = i.v. bolus dose (0.5 µg/kg of BW, diluted in 5 mL of 0.9% sterile saline) of bacterial LPS (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO), or 2) CON (n = 4) = 5-mL i.v injection of 0.9% sterile saline. Treatments were administered on d 0. Average hay DMI during the 5 d preceding treatment application served as covariate within each respective analysis. A treatment × day interaction was detected ($P < 0.01$). Within day; * $P = 0.03$, ** $P < 0.01$.

TABLE 1. Rumen liquid dilution rate, rumen liquid volume, and ruminal in situ disappearance parameters of mixed alfalfa-grass hay in steers assigned to LPS (n = 4) or CON (n = 4)[1]

Item	CON	LPS	SEM	*P* =
Liquid dilution rate, %/h	11.0	8.9	0.6	0.05
Liquid volume, mL/kg of BW	207	88	23	0.01
Disappearance rate, %/h				
DM	12.3	7.1	1.3	0.03
NDF	7.0	4.2	0.6	0.03
Effective degradability,[2] %				
DM	57.7	55.1	0.8	0.05
NDF	67.9	66.5	0.3	0.01

[1]Treatments were 1) LPS = i.v. bolus dose (0.5 µg/kg of BW, diluted in 5 mL of 0.9% sterile saline) of bacterial lipopolysaccharide (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO), or 2) CON = 5 mL i.v. injection of 0.9% sterile saline.

[2]Calculated by fixing ruminal passage rate at 0.046/h (Poore et al., 1990) and using the model proposed by Ørskov and McDonald (1979).

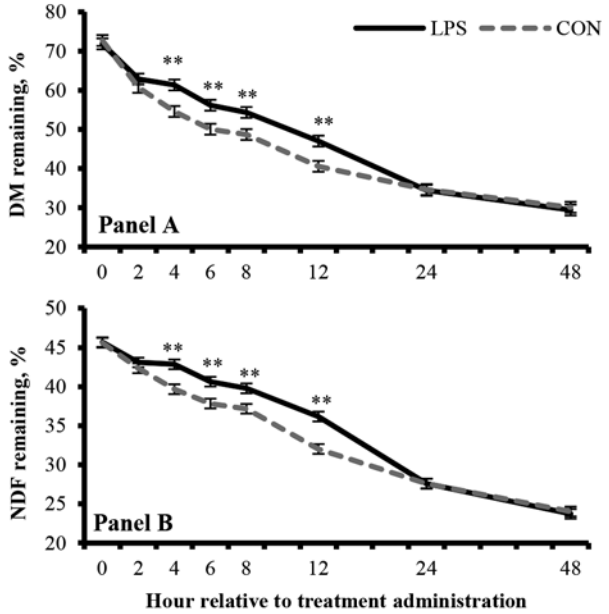

Figure 2. Ruminal hay DM (Panel A) and NDF (Panel B) disappearance, as % remaining of initial DM and NDF content, of steers receiving: 1) LPS (n = 4) = i.v. bolus dose (0.5 µg/kg of BW, diluted in 5 mL of 0.9% sterile saline) of bacterial lipopolysaccharide (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO), or 2) CON (n = 4) = 5 mL i.v. injection of 0.9% sterile saline. Treatments were administered at h 0. Treatment × hour interactions were detected ($P \leq 0.02$). Within hour; ** $P < 0.01$.

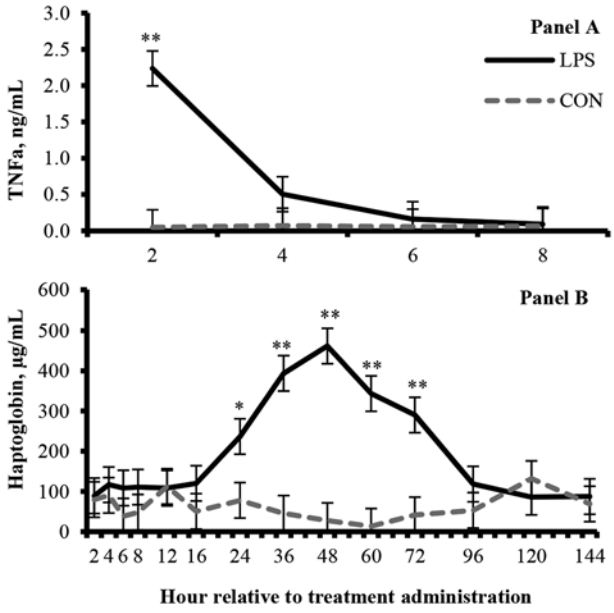

Figure 3. Plasma TNFa (Panel A) and haptoglobin (Panel B) concentrations of steers receiving: 1) LPS (n = 4) = i.v injection (0.5 µg/kg of BW, diluted in 5 mL of 0.9% sterile saline) of bacterial lipopolysaccharide (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO), or 2) CON (n = 4) = 5 mL i.v. injection of 0.9% sterile saline. Treatments were administered at h 0. Values obtained prior to treatment application (-2 and 0 h) served as covariates within each analysis. Treatment × hour interactions were detected ($P < 0.01$). Within hour; * $P \leq 0.05$, ** $P < 0.01$.

reduced by LPS administration despite a potential decrease in ruminal motility and passage rate.

Steers receiving LPS had greater ($P < 0.01$) plasma TNFα concentration at 2 h, and greater ($P \leq 0.05$) plasma haptoglobin concentrations from 24 to 72 h relative to treatment administration compared with CON steers (Figure 3). Steers receiving LPS had greater ($P \leq 0.05$) plasma cortisol concentrations from 2 to 16 h, and greater ($P < 0.01$) serum NEFA concentrations from 6 to 48 h relative to treatment administration compared with CON steers (Figure 4). Steers receiving LPS also had greater ($P < 0.01$) plasma insulin and glucose concentration at 2 h, but reduced ($P \leq 0.05$) plasma glucose concentrations from 4 to 12 h relative to treatment administration compared with CON steers (Figure 4). Steers receiving LPS had greater ($P < 0.01$) plasma cholecystokinin concentration at 16 h, and greater ($P \leq 0.05$) plasma leptin concentrations at 8, 12, 16, 36, and 48 h relative to treatment administration compared with CON steers (Figure 4).

Treatment differences reported for plasma TNFα, haptoglobin, and cortisol responses were expected based on neuroendocrine and innate immune response elicited by LPS administration (Rodrigues et al., 2015b). Supporting treatment effects on hay DMI, TNFα is known to reduce feed intake by modulating the central nervous system and inhibiting digestive function (Klasing and Korver, 1997), whereas plasma haptoglobin concentrations are negatively associated with DMI (Araujo et al. 2010). Treatment differences in serum NEFA concentrations should be mainly attributed to increased lipolysis caused by the LPS-induced cortisol response. Changes in circulating insulin and glucose concentrations upon a LPS stimuli have also been reported by others (Waldron et al., 2003). Collectively, circulating concentrations of insulin, glucose, and NEFA have been associated with feed intake regulation in ruminants, particularly via the hepatic oxidation theory with glucose and NEFA serving as oxidative substrates and suppressing feeding behavior in the brain (Foster et al., 1991; Allen, 2000; Allen et al., 2009). Moreover, leptin synthesis by adipocytes is increased during an inflammatory response (Rodrigues et al., 2015a). Both leptin and cholecystokinin are known to synergistically limit gastrointestinal motility, resulting in satiety and reduced voluntary feed intake (Matson and Ritter, 1999). Hence, the transient decrease in DMI caused by LPS administration can attributed, at least partially, to altered circulating concentrations of hormones and metabolites that modulate gastrointestinal motility and satiety centers in the central nervous system (Klasing and Korver, 1997; Allen et al., 2009).

As previously noted, reduced ruminal motility and feed passage rate should result in increased ruminal degradability of hay DM and NDF (Farooq et al., 2010). Yet, treatment effects on ruminal degradability

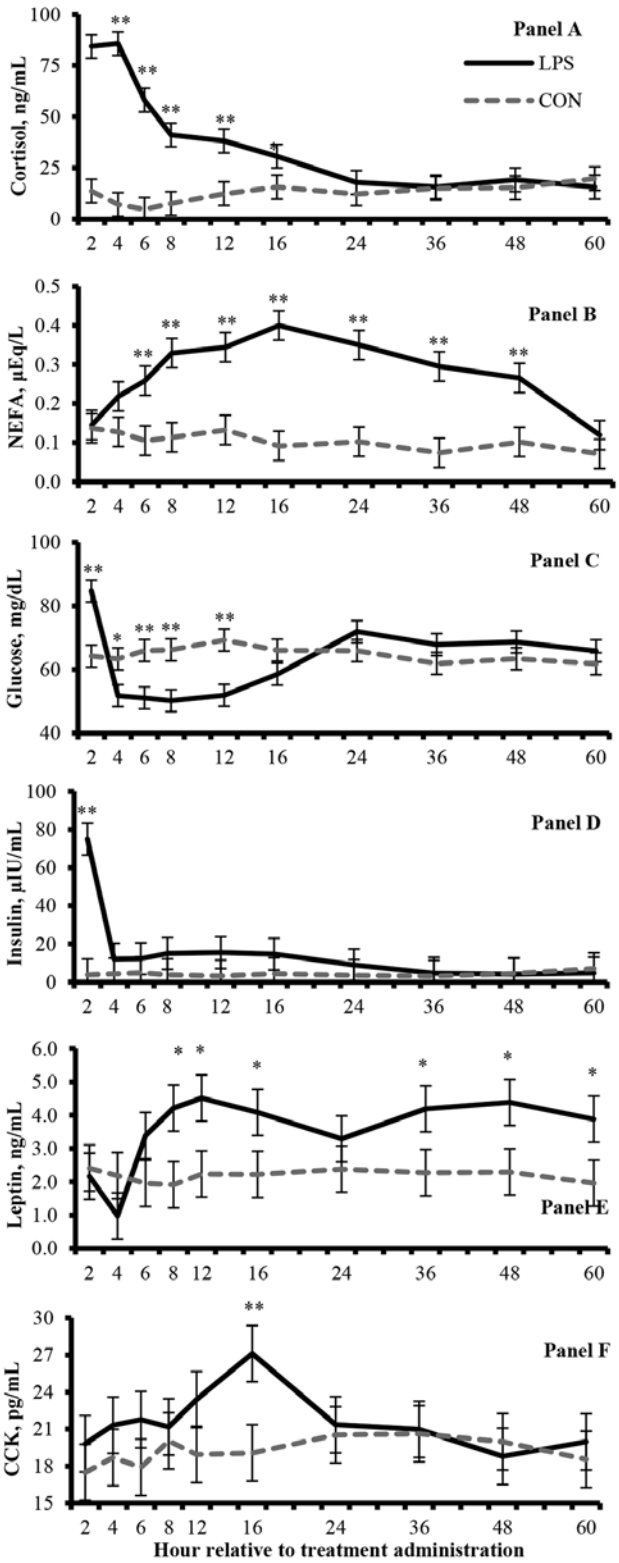

Figure 4. Concentrations of plasma cortisol (Panel A), serum NEFA (Panel B), plasma glucose (Panel C), plasma insulin (Panel D), plasma leptin (Panel E), and plasma cholecystokinin (CCK; Panel F) of steers receiving: 1) LPS (n = 4) = i.v. injection (0.5 µg/kg of BW into 5 mL of 0.9% sterile saline) of bacterial lipopolysaccharide (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO), or 2) CON (n = 4) = 5 mL i.v. injection of 0.9% saline. Treatments were administered at h 0. Values obtained prior to treatment application (-2 and 0 h) served as covariates within each analysis. Treatment × hour interactions were detected ($P \leq 0.09$). Within hour; * $P \leq 0.05$, ** $P < 0.01$.

of hay DM and NDF suggest that LPS administration transiently impaired feed degradability by rumen microbes. During stress and inflammatory challenges, salivary concentrations of cortisol and pro-inflammatory cytokines are immediately increased (Escribano et al., 2014) and are expected to promptly reach the rumen. The ruminal epithelium expresses a variety of immunological receptors and cytokines, which initiate local and systemic inflammatory responses (Trevisi et al., 2013). Moreover, rumen microbes express a variety of receptors that are believed to interact with mammalian hormones, particularly catecholamines (Freestone et al., 2008). Although catecholamines were not evaluated herein, LPS administration is known to sharply increase circulating concentrations of these hormones in cattle (Burdick et al., 2011), which in turn are also known to reach the saliva (Kennedy et al., 2001). Hence, it can be speculated that the neuroendocrine and inflammatory compounds elicited by LPS administration reach the rumen and interact with ruminal and microbial cells. In turn, these compounds may elicit host-microbiome interactions that transiently impair the ability of ruminal microbes to attach and degrade feed particles, as suggested by LPS effects on hay DM and NDF degradability (Table 1; Figure 2).

IMPLICATIONS

Lipopolysaccharide administration transiently reduced feed intake in cattle via physiological, inflammatory, and acute-phase reactions that modulate gastrointestinal motility and satiety centers in the central nervous system, in addition to potential host-microbiome endocrine interactions that impaired ruminal forage degradability. Although research is warranted to validate this latter outcome, this experiment provides novel knowledge that may serve as foundation for future studies investigating the mechanisms by which stress and inflammation impact voluntary feed intake in cattle.

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Heifer development using stockpiled, dormant native forages and protein supplementation delays body weight gain without altering reproductive performance

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ABSTRACT: The objective of this study was to determine the effect of protein supplementation strategy and stockpiled forage type on growth, nutritional status, and reproductive performance of yearling heifers. Spring-born, beef heifers (n = 224) were stratified by BW at weaning to 1 of 3 stockpiled forages: (1) endophyte-infected tall fescue (TF; 7.21% CP and 67.13% NDF, DM basis) (2) big bluestem and indiangrass combination (BI; 4.32% CP and 71.06% NDF, DM basis), or (3) switchgrass (SG; 3.87% CP and 76.79% NDF, DM basis). Forage treatments were then randomly assigned to receive 1 of 2 supplement types: (1) 0.68 kg·heifer^{-1}·d^{-1} of dried distillers grains with solubles (DDGS: 28% CP) or (2) 0.22 kg·heifer^{-1}·d^{-1} of blood meal and fish meal (BF: 72.5% CP), resulting in a 3 × 2 factorial arrangement of treatments. Each year, treatments were initiated in January and terminated in April at the onset of a 60-d breeding season. Heifer BW was recorded monthly until breeding and at final pregnancy diagnosis. Initial BW was not different ($P \geq 0.30$) by forage or supplement type. However during the rest of the study, BW was greater ($P < 0.01$) for TF heifers. From study initiation to breeding, ADG was greater ($P < 0.01$) for TF heifers. However, ADG was greater ($P < 0.01$) for BI and SG heifers from breeding to final pregnancy diagnosis. Heifers grazing TF pastures had greater ($P < 0.01$) overall ADG than heifers grazing warm-season forages. Due to an increased ADG from initiation to breeding of the study, TF heifers had a greater ($P < 0.01$) percent of mature BW (MBW) at breeding for TF heifers. Heifer BW or ADG was not influenced ($P \geq 0.13$) by supplementation strategy during the entire study. Serum glucose concentrations were not different ($P \geq 0.30$) among forage type or supplement strategy. Insulin concentrations exhibited ($P = 0.04$) a forage type × supplement type interaction. Heifers grazing TF had lower ($P < 0.01$) NEFA concentrations. Circulating SUN concentrations were greater ($P < 0.01$) in TF heifers. Final pregnancy rates were not impacted ($P \geq 0.31$) by forage and supplement type. Ultimately, heifers grazing low-quality, warm-season grasses delayed BW gain prior to the breeding season, which did not impact overall pregnancy rates.

Key words: beef heifers, heifer development, reproductive performance
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INTRODUCTION

Extending grazing through the winter utilizing stockpiled forages may be an economical alternative to feeding harvested feedstuffs during heifer development. However, a concern is that heifer BW gain may be insufficient prior to breeding while consuming stockpiled tall fescue (Poore et al., 2006). Additionally, grazing low-quality, warm-season forages may provide another opportunity to develop heifers using stockpiled forages. Supplementation with high RUP increased ADG and may improve energy utilization of heifers grazing low-quality forage (Lalman et al., 1993). In addition, heifers grazing low-quality, dormant range fed a high RUP supplement increased ADG during breeding, increased pregnancy rates, and increased herd retention rate compared to a low RUP supplement (Mulliniks et al., 2013). Furthermore, recent research emphasis has established that developing heifers to a lighter target BW reduced input costs without impairing reproductive function (Roberts et al., 2009; Funston and Larson, 2011; Mulliniks et al., 2013). Thus, we hypothesized that heifers grazing low-quality, warm-season forages would have a compensatory gain period and improve nutrient status before breeding to achieve similar pregnancy rates as heifers grazing cool-season forage. Our objectives

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were to determine the effect of stockpiled winter forage and protein supplementation strategy on BW gain, BCS, serum metabolites, and reproductive performance in yearling beef heifers.

MATERIALS AND METHODS

All animal handling and experimental procedures described were approved by the University of Tennessee, Knoxville Institutional Animal Care and Use Committee.

Spring-born, British crossbred heifers (325.91 ± 2.35 kg at weaning; n = 224) were stratified by BW at weaning and randomly assigned to 1 of 3 forage treatments: (1) endophyte-infected tall fescue (**TF**; *Festuca arundinacea*; 7.21% CP, 67.13% NDF, DM basis), (2) big bluestem (*Andropogon gerardi* Vitman) and indiangrass (*Sorghastrum nutans* L.) combination (**BI**; 4.32% CP, 71.06% NDF, DM basis), or (3) switchgrass (**SG**; Panicum virgatum L.; 3.87% CP, 76.79% NDF, DM basis). Forage treatments then were randomly assigned to receive 1 of 2 supplement types: (1) 0.68 kg·heifer^{-1}·d^{-1} of dried distillers grains with solubles (**DDGS**: 28% CP, 74% RUP, 88% TDN) or (2) 0.22 kg·heifer^{-1}·d^{-1} of blood meal and fish meal (**BF**: 72.5% CP, 67.5% RUP, 69.5% TDN). Treatments were designed as a 3 × 2 factorial arrangement of treatments. Heifers were managed during the development period at the Middle Tennessee Research and Education Center located in Spring Hill, TN.

Heifer BW and BCS (1 = emaciated, 9 = obese; Wagner et al., 1988) were recorded at the initiation of the study and ascertained approximately every 28 d until the end of the breeding season in May and at final pregnancy diagnosis in September. Percent of mature BW at breeding was estimated by the average cow BW of the herd at 5 yr of age for each development treatment. The different developmental treatments were terminated at the onset of the breeding season when heifers were managed together. The breeding season began in April every year and all heifers were synchronized utilizing a controlled internal drug-releasing (CIDR) device (Eazi-Breed CIDR, Zoetis Inc., Kalamazoo, MI) with a 7 d CO-Synch protocol. Heifers received a single 2 mL intramuscular injection of GnRH (Cystorelin, Merial) and a CIDR on -7 d. The CIDR was removed on 0 d and the heifers were administered a 5 mL injection of PGF (Lutelyse, Zoetis Inc., Kalamazoo, MI) intramuscularly. Approximately 66 h after CIDR removal, all cows were given an intramuscular injection of 2 mL of GnRH (Cystorelin, Merial) and artificially inseminated. Finally, fourteen days after TAI, cleanup bulls were utilized to provide natural service to the heifers for a 60 d breeding season with a heifer-to-bull ratio of 1:30. Pregnancy diagnosis occurred for assessment of reception to TAI 30 d after insemination via transrectal ultrasonography. A final pregnancy diagnosis was administered by transrectal ultrasonography in September of every year.

Serum Metabolite Assays. A blood sample (~ 9 mL; Corvac, Sherwood Medical, St. Louis, MO) was collected via coccygeal venipuncture twice prior to the start of breeding to determine nutritional status. Serum samples were analyzed for non-esterified fatty acids (NEFA), urea N (SUN), glucose, β-hydroxybutyrate (BHB), and insulin concentrations. Commercial kits were utilized to perform the analysis for NEFA (Wako Chemicals, Richmond, VA), SUN (Thermo Scientific, Middletown, VA), and glucose (enzymatic endpoint, Thermo Scientific, Middletown, VA). Serum samples of BHB (BHBA (D-(-)-3- Hydroxybutyrate)) were analyzed using a Tris Buffer (10 ml of Tris hydrochloric acid + 40 ml of deionized water, pH 9) with 30 mg of β-Nicotinamide adenine dinucleotide (β-NAD) and an enzyme of 3-hydroxybutyrate dehydrogenase. Concentrations of serum insulin were determined by radioimmunoassay (RIA) (EMD Millipore's Porcine Insulin RIA) using Wizard2 Gamma Counter (Perkin Elmer, Waltham, MA). Inter- and intra-assay CV were less than 10%.

Statistical Analysis. Measurements of heifer performance and serum metabolites were analyzed as a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC, USA). These data were analyzed with pasture as the experimental unit with the fixed effects of forage type, supplement type, year, and their interactions. Repeated measures was utilized for variables collected over time with productive period as the repeated factor. Least squares means were compared using Fisher's LSD at a significance level of $P \leq 0.05$. Binomial data (pregnancy rate) were analyzed with PROC GLIMMIX using a model that included the fixed effects of forage type, supplement type, year, and their interactions.

RESULTS AND DISCUSSION

Circulating glucose concentrations were not different ($P \geq 0.30$; Table 1) among forage types and supplement strategies. Insulin concentrations exhibited ($P = 0.04$) a forage type × supplement type interaction. Heifers grazing BI and supplemented BF had greater ($P = 0.02$) insulin concentrations than BI heifers fed DDGS. However, SG heifers fed BF had lower ($P = 0.03$) circulating insulin than SG heifers fed DDGS. Insulin concentrations may be attributed to differences in nutrient intake of warm-season grasses. An increase in nutrient intake has been reported to increase circulating insulin concentrations (Yelich et al., 1996). Thus, the increase in circulating insulin in the current study may be due to differences in forage intake. Heifers grazing TF had lower (*P*

TABLE 1. Impact of forage type and supplement type on serum metabolites of heifers during the winter grazing period

	Forage Treatment									
	TF[1]		BI		SG			*P*-value		
Measurement	BF[2]	DDGS	BF	DDGS	BF	DDGS	SEM	Forage	Supp	Forage × Supp
Glucose, mg/dl	78.1	75.6	74.5	75.5	78.3	78.0	2.1	0.30	0.71	0.68
Insulin, ng/mL	0.47	0.31	0.58	0.20	0.24	0.42	0.11	0.83	0.20	0.04
NEFA, mmol/L	282	294	380	401	408	457	23	< 0.01	0.11	0.64
BHB, µmol/L	303	279	323	267	305	301	13	0.61	0.01	0.12
SUN, mg/dl	13.2	11.9	10.4	9.1	12.5	9.6	0.5	< 0.01	< 0.01	0.07

[1]Forage: tall fescue (TF), big bluestem and indiangrass combination (BI), and switchgrass (SG).

[2]Supplement: blood meal and fish meal (BF), and dried distillers grains and solubles (DDGS).

Standard error of mean (SEM).

< 0.01) NEFA concentrations than their forage counterparts. Heifers grazing warm-season grasses experienced compensatory gain during the breeding season. Heifers that were feed restricted to maintain BW for 95 d had NEFA concentrations that decreased within 10 d of realimentation to similar levels as control heifers fed ad libitum (Yambayamba et al., 1996). Supplementation strategy had no impact ($P = 0.11$) on circulating NEFA concentrations. Concentrations of BHB were not different ($P = 0.61$) between forage types. Heifers supplemented with BF had greater ($P = 0.01$) BHB concentrations. Circulating SUN concentrations exhibited a tendency ($P = 0.07$) for a forage type × supplement type interaction. Circulating SUN concentrations were greater ($P < 0.01$) in TF and BF-supplemented heifers compared to their respective counterparts. However, heifers grazing BI and supplemented with BF had lower ($P < 0.01$) circulating SUN than TF and SG heifers supplemented with BF. Concentrations of SUN can provide an indication of N availability resulting from deamination of dietary and endogenous protein sources (Roseler et al., 1993). Ruminal N recycling may preserve dietary N in response to nutrient restriction (Bunting et al., 1989), and compensatory gain following nutrient restriction may improve metabolic and N efficiency (Freetly and Nienaber, 1998). Therefore, heifers grazing warm-season grasses may have increased N utilization efficiency and spared protein as an energy source during the breeding season.

Initial BW was not different ($P \geq 0.30$; Table 2) for heifers by forage type or supplementation strategy. Body weight was greater ($P < 0.01$) for TF heifers at breeding, AI pregnancy diagnosis, and final pregnancy diagnosis compared to BI and SG heifers. Heifer BW was not influenced ($P \geq 0.16$) by supplementation strategy. From study initiation to breeding, heifer ADG was greater ($P < 0.01$) for TF heifers compared to BI and SG heifers. However, heifers grazing BI and SG pastures compensated from the restricted gain and had greater ($P < 0.01$) ADG from breeding to final pregnancy diagnosis than heifers grazing TF. Overall, TF heifers had greater ($P < 0.01$) ADG than their forage counterparts from study initiation to final pregnancy diagnosis. In addition, supplementation strategy had no impact ($P \geq 0.13$) on ADG during the entire study.

Percent of mature BW (MBW) at breeding was greater ($P < 0.01$; Table 2) for TF heifers compared to SG and BI heifers. In addition, heifers supplemented with BF tended to have greater ($P = 0.07$) MBW at breeding than DDGS heifers. Patterson et al. (1992) established that heifers should attain 60-65% of MBW prior to breeding to optimize reproductive success. However, heifers developed to a lower (53%) mature BW had similar reproductive performance to heifers raised to greater (58%) mature BW (Funston and Deutscher, 2004). Additionally, heifers grazing dormant range and fed 50% RUP reached 51% MBW while achieving a 94% pregnancy rate (Mulliniks et al., 2013). Therefore, developing heifers to a lower target BW may not impact reproductive performance.

Heifer BCS was not different ($P > 0.27$; Table 2) by forage type or supplement type at the initiation of treatment. However, TF heifers had greater ($P < 0.01$) BCS at breeding and greater ($P < 0.01$) BCS at final pregnancy diagnosis. Supplementation strategy had no impact ($P \geq 0.27$) on BCS during the entire study.

Pregnancy rates at TAI exhibited a tendency ($P = 0.06$; Table 2) for a forage type × supplement type interaction. Heifers grazing TF and supplemented with DDGS tended to have greater ($P = 0.07$) pregnancy rates than BF heifers. However, heifers grazing BI and SG had no difference in TAI pregnancy rates ($P \geq 0.12$) based on supplementation strategy. Final pregnancy rates were not impacted ($P \geq 0.31$) by forage type or supplementation strategy. In the present study, heifers grazing warm-season grasses had improvements in gain post-breeding that may have influenced reproductive performance. In support, heifers that had an improved plane of nutrition during the first 21 d post-AI had greater pregnancy rates when compared with heifers that maintained or lost BW (Arias et al., 2012). Overall, reproductive performance

TABLE 2. Forage type and supplement type effects on heifer growth and reproductive performance during the winter grazing period

	Forage Treatment									
	TF[1]		BI		SG			*P*-value		
Measurement	BF[2]	DDGS	BF	DDGS	BF	DDGS	SEM	Forage	Supp	Forage × Supp
n =	37	41	37	31	42	36				
BW, kg										
Initial	326	322	326	325	328	325	4	0.72	0.30	0.82
Breeding	358	350	328	322	304	308	3	< 0.01	0.16	0.14
AI Pregnancy Diagnosis	384	379	362	360	344	346	4	< 0.01	0.55	0.62
Final Pregnancy Diagnosis	434	432	417	413	400	402	3	< 0.01	0.62	0.58
ADG, kg/d										
Initial to Breeding	0.38	0.29	0.04	-0.03	-0.22	-0.19	0.04	< 0.01	0.16	0.11
Breeding to final pregnancy diagnosis	0.65	0.70	0.76	0.80	0.82	0.82	0.02	< 0.01	0.13	0.51
Initial to final pregnancy diagnosis	0.56	0.55	0.47	0.44	0.37	0.39	0.02	< 0.01	0.67	0.46
Percentage of mature BW, %	55.5	53.7	50.8	49.6	47.3	47.4	0.7	< 0.01	0.07	0.31
BCS										
Initial	5.78	5.81	5.84	5.66	5.81	5.79	0.06	0.68	0.27	0.26
Breeding	5.68	5.66	5.43	5.38	5.30	5.37	0.07	< 0.01	0.94	0.63
Final pregnancy diagnosis	5.81	5.90	5.65	5.61	5.61	5.70	0.07	< 0.01	0.41	0.51
Reproductive Performance										
AI Pregnancy Rate, %	47	66	57	42	50	55	8	0.64	0.67	0.06
Final Pregnancy Rate, %	92	93	89	97	90	92	5	0.78	0.31	0.59

[1]Forage: tall fescue (TF), big bluest

was not impacted by grazing low-quality forage, which may be partially explained by the greater ADG from breeding to final pregnancy diagnosis of heifers grazing warm-season grasses. In the present study, overall pregnancy rates were not different with heifers ranging from 47 to 55% MBW at the start of breeding. Thus, developing heifers to a lower target BW did not negatively impact reproductive performance.

IMPLICATIONS

This study indicated that heifers can be developed at a slow rate of gain grazing low-quality, warm-season forages using high-RUP supplementation without negatively impacting reproductive performance. Development on warm-season grasses may have resulted in an increase in efficiency of gain and improvement of nutrient utilization during the breeding season. Overall, supplementation strategy with a plant- or animal-based RUP supplement had little impact on heifer growth and reproductive performance.

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Moderate nutrient restriction influences transcript abundance of genes impacting production efficiencies of beef cattle in fetal liver, muscle, and cerebrum by d 50 of gestation[1]

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ABSTRACT: We hypothesized that a moderate maternal nutrient restriction during the first 50 d of gestation in beef heifers would affect transcript abundance of genes impacting production efficiency phenotypes in fetal liver, muscle, and cerebrum. Fourteen Angus-cross heifers were estrus synchronized and assigned at breeding to one of two dietary treatments (CON- 100% of nutrient requirements to gain 0.45 kg/d; RES- 60% of CON). At d 50 of gestation, heifers were ovariohysterectomized, and fetal liver, muscle, and cerebrum were collected. Analysis for RNA-seq was conducted on the Illumina HiSeq 2500 platform using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample. Transcriptome analysis was performed using the Tuxedo Suite, and ontological analysis with DAVID 6.8. For fetal liver, muscle, and cerebrum, a total of 548, 317, and 151 genes, respectively ($P < 0.01$) were differentially expressed, of which 201, 144, and 28 genes, respectively were false discovery rate protected (FDR; $q < 0.10$). Differentially expressed genes were screened to determine whether they fit into functional categories of pathways or ontologies associated with known impacts on production efficiencies. In fetal liver, 5 functional categories of interest were affected by nutritional treatment: metabolic pathways (n = 43 genes), protein kinase (n = 47 genes), nucleosome core (n = 22 genes), mRNA splicing (n = 7 genes), and complement/coagulation cascades (n = 6 genes). In fetal muscle, 3 functional categories of interest were affected by nutritional treatment: skeletal muscle (n = 74 genes), embryogenesis (n = 14 genes), and signaling cascades (n = 18 genes). In fetal cerebrum, 3 functional categories of interest were affected by nutritional treatment: hippocampus and neurogenesis (n = 32 genes), metal-binding (n = 23 genes), and cytoskeleton (n = 5 genes). These results demonstrate that a moderate maternal nutrient restriction during the first 50 d of gestation in beef heifers alters transcript abundance of genes impacting production efficiencies in fetal liver, muscle, and cerebrum. Additionally, these data lay the foundation upon which further research identifying the phenotypic responses to changes in these pathways may be elucidated. Finally, identifying specific supplementation strategies to mitigate alterations in transcript abundance due to aberrant maternal nutrition during early gestation will provide additional means to increase production efficiencies in beef cattle. Emerging targets include liver metabolism and feed efficiency, muscle fiber formation and tenderness, as well as programmed cerebral formation and temperament.

Key words: developmental programming, fetus, nutrition, RNA-Seq
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INTRODUCTION

Research investigating developmental programming, or the phenomenon in which maternal metabolic state, physiological traits, or environmental factors, influence fetal growth and development leading to permanent changes in postnatal physiology (Barker

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and Clarke, 1997), has elucidated the importance of the uterine environment for the developing fetus. As 75% of the prenatal growth of the ruminant fetus occurs during the last 2 mo of gestation (Robinson et al., 1977), and nutrient requirements for fetal growth and development during the first one-half of gestation are minimal compared with late gestation (Funston et al., 2010), the majority of fetal development research has been focused on the latter half. During the early phase of fetal development, however, differentiation and vascularization of utero-placental tissues, as well as fetal organogenesis occur, all of which are critical events for normal fetal development (Funston et al., 2010). Additionally, dams that undergo stress (nutritional, environmental, etc.) during the beginning of gestation but not the end are likely to produce a normal birth weight offspring that may still suffer from poor growth and metabolic issues because of the stress early in pregnancy (Ford et al., 2007; Vonnahme et al., 2007; Reynolds and Caton, 2012). These stress-induced phenotypic changes may arise by affecting gene transcript abundance in fundamental production tissues such as liver, muscle, and brain, thus "programming" potential susceptibilities to metabolic issues and reduced performance (Waterland and Jirtle, 2004). Therefore, we hypothesized that a moderate maternal nutrient restriction during the first 50 d of gestation in beef heifers would affect gene transcript abundance in fetal liver, muscle, and cerebrum thereby programming susceptibilities to impaired performance and altered carcass characteristics postnatally.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Angus-cross heifers (n = 14, ~16 mo of age; average initial BW = 313 ± 24.9 kg) were obtained from the Central Grasslands Research and Extension Center (Streeter, ND); and housed at the NDSU Animal Nutrition and Physiology Center (Fargo, ND). Heifers were acclimated to individual bunk feeding (American Calan, Northwood, NH) for 2 wk before the beginning of the trial. All heifers were exposed to the 5-d CO-Synch + CIDR estrus synchronization protocol (Bridges et al., 2008) and bred via AI to a common sire at 12 h after observed estrus. Immediately post-breeding, heifers were randomly assigned to one of two treatment groups. Control heifers (CON, n = 7), received 100% of NRC (2000) requirements for 0.45 kg/d gain to reach 80% of mature BW at first calving (Actual ADG = 0.51 kg/d). Restricted heifers (RES, n = 7), were placed on a 40% global nutrient restriction, which was accomplished by reducing total diet delivery to 60% of the control delivery (Actual ADG = -0.08 kg/d). The diet was delivered via TMR (48.37% DM, 5.28% CP, 6.77% ash, and 29.43% NDF on a DM basis) and consisted of grass hay, corn silage, alfalfa haylage, as well as grain and mineral mix. Dried distillers grains with solubles (53.4% NDF, 31.3% CP) were supplemented in addition to the TMR to meet protein requirements of individual heifers.

Tissue Collection and Analysis

Ovariohysterectomy procedures were conducted as described by McLean et al. (2016) on d 50 of gestation for all heifers. Following ovariohysterectomy, fetal liver, muscle from the hind limb, and cerebrum tissues were collected using a stereoscope for increased visualization and to ensure maximum yield of tissue. Once collected, all tissues were snap frozen in liquid nitrogen-cooled isopentane (2-Methylbutane; J.T.Baker, Center Valley, PA) and stored at -80°C. RNA was extracted and RNA-seq analysis was conducted on the Illumina HiSeq 2500 platform using 50-bp paired-end reads at a depth of 2 × 10.4M reads/sample by the University of Minnesota Genomics Center (Minneapolis-St. Paul, MN). Transcriptome analysis was performed using the Tuxedo Suite (Trapnell et al., 2012), and KEGG pathways as well as gene ontologies, which identify groups of genes with similar functions, were analyzed with DAVID 6.8 (Huang et al., 2009a,b). Individual gene significance was set at $q \leq 0.10$ (equivalent to $P < 0.00035$), and all genes within tissue type that were $P \leq 0.01$ were used for analysis with DAVID 6.8 to determine ontologies of interest. For fetal liver, muscle, and cerebrum, a total of 548, 317, and 151 genes ($P < 0.01$) were differentially expressed, of which 201, 144, and 28 genes were false discovery rate protected (FDR; $q < 0.10$). Differentially expressed genes were screened to determine whether they fit into functional categories of pathways or ontologies associated with known impacts on production efficiencies.

RESULTS

Liver

Five functional categories of interest were determined for fetal liver tissues: metabolic pathways, protein kinase, nucleosome core, mRNA splicing, and complement/coagulation cascades (Table 1). The metabolic pathways category (n = 43 genes; $P = 0.017$) comprised 6 proposed functions (Table 1): Amino acid me-

TABLE 1. Functional categories and predicted roles for differentially expressed genes that impact production efficiencies ($P < 0.01$) in fetal liver, muscle from hind limb, and cerebrum

Tissue	Category	Functional annotation[1]	Total genes[2]	RES[3]	CON[4]	*P*-value[5]
Liver	Metabolic pathways	Amino acid	10	5	5	0.017
		Purine and pyrimidine	7	7	0	
		Carbohydrate	10	5	5	
		Reducing equivalent (NAD/FAD)	5	5	0	
		Steroid and lipid biosynthesis	9	8	1	
		Cytochrome and heme	2	2	0	
	Protein kinase	Serine/Threonine protein kinase	22	21	1	0.020
		ATP-binding	19	15	4	
		Nucleotide-binding	6	4	2	
	Nucleosome core	Histones	9	9	0	0.005
		Histone modifiers	13	12	1	
	mRNA splicing	Spliceosome	7	6	1	0.041
	Complement/ Coagulation	Complement factors	3	3	0	0.041
		Coagulation factors	3	3	0	
Muscle	Skeletal muscle	Contraction	9	9	0	< 0.001
		Intermediate filament	11	7	4	
		Microtubule	10	2	8	
		Actin	4	3	1	
		Myosin	4	4	0	
		Troponin	6	6	0	
		Calcium-binding	25	14	11	
		ATP-binding	5	0	5	
	Embryogenesis	Myogenesis	2	2	0	< 0.001
		Homeobox	12	10	2	
	Signaling cascades	Wnt	6	4	2	0.003
		MAPK	12	3	9	
Cerebrum	Hippocampus and neurogenesis	Hippo signaling pathway	5	5	0	< 0.001
		Collagen	9	9	0	
		Netrin	5	5	0	
		SMAD	4	4	0	
		Developmental protein	9	8	1	
	Metal-binding	Iron-binding	4	4	0	0.006
		Zinc-binding	10	10	0	
		Copper-binding	2	2	0	
		Nickel-binding	1	1	0	
		Calcium-binding	6	5	1	
	Cytoskeleton	Actin remodeling	5	5	0	0.003

[1]Proposed function of differentially expressed genes that fall under a specific category.

[2]Total number of differentially expressed genes associated with a specific function.

[3]Number of differentially expressed genes that are upregulated in RES fetuses.

[4]Number of differentially expressed genes that are upregulated in CON fetuses.

[5]Probability value associated with a specific category.

tabolism (n = 10) was made up of 5 genes upregulated in RES, and 5 upregulated in CON; all differentially expressed purine and pyrimidine metabolism genes (n = 7) were upregulated in RES; carbohydrate metabolism (n = 10) comprised 5 genes upregulated in RES, and 5 upregulated in CON; all differentially expressed reducing equivalent metabolism genes (n = 5) were upregulated in RES; steroid and lipid biosynthesis (n = 9) were affected by treatment such that 8 genes were upregulated in RES and 1 was upregulated in CON; and cytochrome and heme metabolism (n = 2) were affected by treatment such that both genes were upregulated in RES. The protein kinase category (n = 47 genes; P = 0.020) comprised 3 proposed functions (Table 1): serine/threonine protein kinase (n = 22) yielded 21 genes that were upregulated in RES, and 1 gene upregulated

in CON; ATP-binding function (n = 19) was made up of 15 genes upregulated in RES, and 4 upregulated in CON; and nucleotide-binding (n = 6) of which 4 were upregulated in RES and 2 were upregulated in CON. The nucleosome core category (n = 22 genes; $P = 0.005$) comprised 2 proposed functions (Table 1): all differentially expressed histones (n = 9) were upregulated in RES; and histone modifiers (n = 13 genes) comprised 12 genes upregulated in RES and 1 gene upregulated in CON. The mRNA splicing category (n = 7 genes; $P = 0.041$) contained 6 genes upregulated in RES, and 1 gene upregulated in CON. The complement and coagulation cascade category (n = 6 genes; $P = 0.041$) comprised 2 functions (Table 1): complement factors and coagulation factors (n = 3 and n = 3, respectively) of which all genes were upregulated RES.

Muscle

Three categories of interest were determined for fetal muscle tissue: skeletal muscle, embryogenesis, and signaling cascades (Table 1). The skeletal muscle category (n = 74 genes; $P < 0.001$) comprised 8 proposed functions (Table 1): contraction genes (n = 9) all of which were upregulated in RES; the intermediate filament genes (n = 11) of which 7 genes were upregulated in RES and 4 upregulated in CON; microtubule associated genes (n = 10) contained 2 genes upregulated in RES and 10 upregulated in CON; actin (n = 4) was made up of 3 genes upregulated in RES and 1 upregulated in CON; all genes associated with myosin and troponin (n = 4 and n = 6 genes, respectively) were upregulated in RES; 25 genes were associated with calcium-binding in skeletal muscle, of which 14 were upregulated in RES and the remaining upregulated in CON; and all differentially expressed ATP-binding genes (n = 5) were upregulated in CON. The embryogenesis category (n = 14 genes; $P < 0.001$) comprised 2 functional ontologies (Table 1): myogenesis (n = 2) of which both genes were upregulated in RES; and homeobox related genes (n = 12) of which 10 were upregulated in RES, and 2 were upregulated in CON. The signaling cascades category (n = 18 genes; $P = 0.003$) was made up of 2 functional ontologies (Table 1): the Wnt signaling pathway (n = 6) had 4 genes upregulated in RES, and 2 genes upregulated in CON; and MAPK pathway (n = 12) comprised 3 genes upregulated in RES and 9 genes upregulated in CON.

Cerebrum

Three categories of interest were determined for fetal cerebrum; hippocampus and neurogenesis, metal-binding, and cytoskeleton (Table 1). The hippocampus and neuro-genesis category (n = 32 genes; $P < 0.001$) comprised 5 proposed functional annotations (Table 1): Hippo signaling pathway, of which all 5 genes were upregulated in RES; all differentially expressed collagen genes (n = 9) were upregulated in RES; netrin genes (n = 5) were all upregulated in RES; genes associated with the SMAD protein (n = 4) were all upregulated in RES; and genes that encompass developmental proteins (n = 9) were made up of 8 genes which were upregulated in RES and 1 gene upregulated in CON. The metal-binding category (n = 23 genes; $P = 0.006$) comprised 5 metal binding functional annotation groups (Table 1): all differentially expressed iron-binding genes (n = 4) were upregulated in RES; all differentially expressed zinc-binding genes (n = 10) were upregulated in RES; copper and nickel binding genes (n = 2 and n = 1, respectively) were all upregulated in RES; and of the calcium-binding genes (n = 6), 5 were upregulated in RES, and 1 was upregulated in CON. The cytoskeleton category (n = 5) was made up of actin remodeling genes, of which all 5 were upregulated in RES.

DISCUSSION

Altering liver function by modifying metabolism in rats from restricted mothers, is reflected by permanent changes in activities of key hepatic enzymes and kinases in a direction which would potentially bias the liver toward a "starved" setting (Desai and Hales, 1997). These modifications in hepatic metabolism were also demonstrated with sheep in that dietary restriction of ewes from d 28 to 78 of gestation influenced liver function of offspring, creating greater hepatic lipid and glycogen content than controls, and modifying glucose metabolism and glucose/insulin homeostasis postnatally (George et al., 2012). These data may be reflected in our differentially expressed metabolism and protein kinase genes, as well as modifications to carbohydrate, amino acid, and reducing equivalent metabolism, which are highly intertwined and may result in the similar metabolic consequences previously observed in sheep and humans. Our findings of altered genes related to core histones are also supported by observations of nutrient restriction in mothers resulting in modification of transcriptional regulators such as core histones in rat pups (Tosh et al., 2010). Histone modification is critical as it can impact gene expression, chromosome packaging, and DNA damage/repair (Wood, 2004).

The fetal stage is crucial for skeletal muscle development in mammalian livestock, because there is no net increase in the muscle fiber number after birth (Stickland, 1978; Zhu et al., 2004) and therefore any

impacts of maternal nutrition during gestation have lifelong consequences. Greenwood et al. (2004) demonstrated that steers born from cows nutritionally restricted during late gestation had reduced BW and carcass weights at 30 mo of age compared with steers from cows fed adequately. Muscle fibers are formed throughout gestation during primary and secondary myogenesis, and at d 50 of gestation peak primary myogenesis is occurring (Yan et al., 2013), with secondary myogenesis taking place during the second and third trimester (Russell and Oteruelo, 1981). Our data suggest that genes involved in skeletal muscle formation and function were altered by maternal nutritional treatment, which may affect total fiber development during gestation. Additionally, affecting genes involved in muscle function, more specifically contraction, may affect muscle function postnatally and potentially tenderness after slaughter.

The hippocampus in the cerebrum of the brain plays an integral part in emotion and memory and is also linked to anxiety (Engin and Treit, 2007). Key functional proteins in the brain such as collagen, actin filaments, and metal- binding proteins play important roles in maintaining proper synapse and neuronal function, which if altered, may lead to brain disorders such as schizophrenia and abnormal startle response (Lamprecht, 2014; Cristóvão et al., 2016; Su et al., 2016). Changes to these cerebrum functions in cattle seen in our data may indicate that poor maternal nutrition during early gestation may program cattle for a flighty temperament, thereby decreasing production efficiencies.

IMPLICATIONS

Data in the current report clearly indicate that moderate global maternal nutrient restriction during the first 50 d of gestation alters transcript abundance of genes impacting production efficiencies in fetal liver, muscle, and cerebrum. Moreover, these data may provide insight into the mechanisms of action by which global maternal undernutrition during the first 50 d of gestation could impact liver metabolism, muscle fiber number and function, as well as the potential for programmed temperament, all of which influence production efficiencies and thus profitability of beef production as a whole. Finally, these data indicate that although 75% of fetal growth occurs during the last 2 mo of gestation, cellular processes can be modified during the first 50 d of gestation, emphasizing the need for further research to elucidate the mechanisms by which such changes in transcript abundance occur during early gestation, and their respective effects on whole animal lifetime performance.

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Effect of nutrient restriction and central administration of beta-hydroxybutyrate on circulating metabolites in ovariectomized ewes

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ABSTRACT: The objective of this study was to evaluate the effect of nutrient restriction and lateral ventricle infusions of exogenous β-hydroxybutyrate (BHB) on circulating serum metabolites in ovariectomized ewes. Twenty-one ovariectomized Suffolk-crossed ewes were individual fed once daily of a 13.5% CP and 72.5% TDN complete diet. Ewes were stratified by BW and randomly assigned to be fed either at BW maintenance (MAINT) or fed at a 30% feed reduction (RES) for 79 d. Ewes were fed their respective dietary treatment for 70 d prior to receiving their lateral ventricle treatments. On 64 d, ewes were fit with intracerebroventricular (ICV) cannulas. On 70 d ewes were randomly assigned to be centrally injected for 10 d with 300 µl into the lateral ventricle twice daily with one of two treatments: (1) β-hydroxybutyric acid sodium salt solution (BHB; 12,800 µmol/L) or (2) saline solution (CON; 0.9% NaCl). Treatments were followed by 400 µl of sterile saline to ensure treatments cleared the catheter. On d 57, 61, 70, 73, 77 jugular blood samples were collected for analysis of serum glucose, insulin, BHB, NEFA, and urea N concentrations. On d 70 and 75, cerebral spinal fluid (CSF) was collected before treatment infusions for evaluation of BHB concentration. A dietary treatment × ICV treatment was not exhibited ($P \geq 0.18$) for any measurement during this study. As designed, BW decreased ($P < 0.01$) with diet restriction in the RES ewes. Likewise, BCS was decreased ($P = 0.02$) in RES ewes. Serum glucose concentrations decreased ($P = 0.02$) with infusion of BHB. Serum insulin concentrations were unaffected ($P = 0.81$) by central infusion of BHB; however, insulin concentrations did decrease ($P < 0.01$) in RES ewes. Endogenous BHB concentration was unaffected ($P \geq 0.73$) by exogenous BHB infusion or dietary treatments. Serum NEFA concentration increased ($P < 0.01$) with RES ewes; however, NEFA concentration was unaffected ($P = 0.74$) by BHB infusion. Serum urea N concentrations increased ($P < 0.01$) in RES ewes; conversely, SUN was unaffected ($P = 0.88$) by infusion of BHB. Beta-hydroxybutryate concentration in the CSF was unaffected ($P \geq 0.49$) by diet and ICV treatment. This study indicates that central infusion of exogenous BHB may be a metabolic signal to alter metabolic homeostasis through regulation of glucose. Additionally, chronic feed restriction may comprise overall metabolic state long term; however, central infusion of BHB did not exacerbate the effects of negative energy balance.

Key words: β-hydroxybutyrate, energy balance, ewes
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INTRODUCTION

Glucose metabolism is one of the most important aspects for metabolic homeostasis in ruminants. If glucose availability is decreased or glucose metabolism is impaired, then the depletion oxaloacetate as a key intermediate for gluconeogenesis can occur (Hawkins et al., 2000). With these impaired metabolic processes and decreases in energy status, increases in body tissue mobilization occur, resulting in increased NEFA concentrations. Increases in NEFA concentrations may exceed the ability of the liver to appropriately oxidize fatty acids to acetyl CoA and subsequently lead to elevated circulating ketones, especially β-hydroxybutyrate (BHB). Elevated concentrations of BHB have been associated with poor adaptation to negative energy balance (NEB) (Herdt, 2000; Mulliniks et al., 2013). Aside from functioning as a metabolic intermediate during periods of metabolic dysfunction, BHB can influence processes related to nutrient utilization both centrally and peripherally (Rojas-Morales et

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al., 2016). The hypothalamus and pituitary are known for playing central roles in the integration of information for energy balance (St-Amand et al., 2011). However, it is unclear if BHB effects on the hypothalamus-pituitary axis are direct affects or the BHB action is through intermediate mechanisms (DiCostanzo et al., 1999). Therefore, BHB may be a mediator of homeorhesis and leads to altered metabolic homeostasis during metabolic dysfunctions and NEB.

The hypothesis for our research was that chronic exogenous infusion of β-hydroxybutyrate in the lateral ventricle of the brain will alter metabolites in ovariectomized ewes and will be exacerbated by nutrient restriction and negative energy balance. Therefore, the objective of this study was to evaluate the effect of nutrient restriction and lateral ventricle infusions of exogenous BHB on circulating serum metabolites in ovariectomized ewes.

MATERIALS AND METHODS

All experimental procedures and animal handling were in agreement with guidelines established and approved by the University of Tennessee's Institution of Animal Care and Use Committee.

Twenty-one yearling Suffolk-crossed ovariectomized ewes were stratified by BW and randomly assigned to be fed to BW maintenance (**MAINT**) or fed at a 30% feed reduction (**RES**) for 79 d. Ewes were individually feed at approximately 0800 h daily of a diet that was 13.5% CP and 72.5% TDN (SDK Laboratories). Feed intake was recorded daily for each individual ewe by recording feed offered and refused. Ad libitum access to water was provided throughout the duration of the study.

On 64 d, ewes were fit with intracerebroventricular cannulas (ICV) into the lateral ventricle of the brain as previously described (Whitlock et al., 2010). On 70 d, ewes were randomly selected to receive a central infusion for 10 consecutive days with 300 µl into the lateral ventricle twice daily (~12 h apart) with one of two treatments: (1) β-hydroxybutyric acid sodium salt solution (**BHB**; 12,800 µmol/L) or (2) saline solution (**CON**; 0.9% NaCl). Following aseptic preparation, ICV treatments were administered through ICV cannulas with a 25 gauge Huber Point needle followed by 400 µl of sterile saline to ensure treatments cleared the catheter. Beta-hydroxybutyrate solution was prepared according to a previously defined method by Zarrin et al. (2013). Daily infusion amounts were aliquoted under a sterile hood into a sterile conical tube to avoid contamination of the ICV cannula. The solution of β-hydroxybutyrate was stored at 4°C until infusion.

Sampling and Analyses. Jugular blood samples (< 9 mls) were collected from ewes on 57 d, 61 d, 70 d, 73 d, and 77 d. Blood samples were collected in Corvac serum separator tubes (Corvac, Sherwood Medical, St. Louis, MO). Blood samples were collected, cooled, and centrifuged at 2,000 × *g* at 4°C for 20 min. Serum samples were collected, poured into conical tubes, and stored at -20°C for subsequent analyses. Cerebral spinal fluid was collected on 70 d and 75 d 12 h post morning infusions, respectively. To account for any remaining sterile saline from the previous treatment in the ICV catheter, 400 µl was first removed and discarded before CSF collection.

Serum samples were analyzed for glucose, insulin, NEFA, BHB, urea N (SUN). Serum samples were analyzed using a 96-well microplate reader spectrophotometer with commercial kits for NEFA (Wako Chemicals, Richmond, VA; sensitivity of 0.01 mmol/L), glucose (Thermo Electron Corp., Waltham, MA; sensitivity of 0.3 mg/dL), and SUN (Thermo Electron Corp., Waltham, MA; sensitivity of 2.0 mg/dL). Endogenous serum BHB concentrations were determined using DL-β-hydoroxybutyric acid sodium salt, β-Nicotinamide adenine dinucleotide hydrate, and 3-hydroxybutyrate dehydrogenase (Sigma-Aldrich, St. Louis, MO) as previously described by McCarthy et al. (2015). Concentrations of serum insulin were determined by RIA (EMD Millipore's Porcine Insulin RIA) with a Wizard2 Gamma Counter (Perkin Elmer, Waltham, MA). The CSF concentrations (µmol/L) were determined using a commercial quantitative sandwich ELISA kit for Beta-Hydroxybutyratic Acid (MyBioSource, San Diego, CA). All assay inter- and intra-assay CV were less than 15%.

Ewes were weighed twice weekly for a weekly average BW. Weekly BW were used to adjust individual ewe feed amounts. In addition, BCS (1-5; Russel et al., 1969) were recorded throughout the duration of the study by two trained technicians.

Statistical Analysis. Data were analyzed using PROC MIXED in SAS (SAS Institute Inc., Cary, NC, version 9.4). Repeated measures in the MIXED procedure were used to analyze serum metabolite data. The repeated measure was day and the models were tested using sheep as the subject term. The models for glucose, insulin, NEFA, and SUN included fixed effects of day, treatment, diet, breed, and treatment by diet interaction. The model for BHB included fixed effects of treatment, diet, diet by treatment interaction, and day by diet interaction. Variance Components structure was determined to be the most desirable covariance structure according the Akaike's information criterion. The data were analyzed using the Kenward-Roger degrees of freedom method. Data are presented as least squares means and differences were considered significant at $P < 0.05$.

TABLE 1. Effect of a nutrient restriction and central injection of exogenous β-hydroxybutyrate (BHB) into the lateral ventricle on circulating serum metabolites[1]

	Diet[2]							
	MAINT		RES			*P*-value		
Measurement	BHB	CON	BHB	CON	SEM	Diet	Treatment	Diet × ICV Infusion
BCS	2.50	2.50	2.24	2.34	2.52	0.02	0.47	0.64
Metabolites								
Glucose, mg/dL	76.31	90.14	80.89	84.48	4.82	0.88	0.02	0.18
Insulin, ng/mL	0.47	0.50	0.31	0.31	0.07	< 0.01	0.80	0.80
BHB, µmol/L	337.41	332.43	336.7	324.42	27.22	0.86	0.73	0.88
NEFA, µmol/L	80.52	86.67	131.98	134.39	16.82	< 0.01	0.74	0.88
SUN[3], mg/dL	12.44	14.57	19.78	18.12	2.06	< 0.01	0.88	0.24

[1]Infustion treatment: A single centrally infusion into the lateral ventricle with 1 mL of either β-hydroxybutyric acid sodium salt solution (BHB; 12,800 µmol/L) or saline solution (CON; 0.9% NaCl)

[2]Diet: Ewes were assigned to one of two diets: maintenance of BW (MAINT) or 30% feed reduction (RES).

[3]SUN: Serum urea N

RESULTS AND DISCUSSION

Ewe BW or BCS did not exhibit ($P \geq 0.64$; Table 1) a diet × ICV infusion interaction. Ewe BW was less ($P < 0.01$) in dietary restricted ewes; however, BW was unaffected ($P = 0.56$) by BHB infusion. Likewise, BCS was decreased ($P = 0.02$) in RES ewes. Due to no differences in BW, BCS was unaffected ($P = 0.47$) by BHB infusion.

Serum glucose concentrations decreased ($P = 0.02$; Table 1*)* with infusion of BHB. In agreement, Park et al. (2011) reported decreased glucose concentrations in male rats centrally infused with BHB. The decrease in glucose concentration may be attributable to a glucose-sparing (Moore et al., 1976; Zarrin et al., 2013). It has been previously reported that during periods of elevated ketones, glucose utilization by peripheral tissues is decreased by 30% (Mebane and Madison, 1962). The central response to ketone bodies may be mediated through the hypothalamus, as the hypothalamus is the primary regulator of energy homeostasis (Park et al., 2011). The decrease in circulating glucose concentrations may also be related to a decrease in glucose production (Shaw and Wolfe, 1984) rather than a change in glucose utilization. Though glucose concentrations were decreased by infusion of BHB, glucose concentrations were unaffected ($P = 0.88$) by feed restriction. Despite feed restriction, glucose remained tightly regulated within both diet groups (Brockman and Laarveld, 1986). Additionally, the glucose stability during feed restriction may be explained by the decrease in insulin concentrations to lower tissue glucose utilization in the Res ewes (Ouellet et al., 2001).

Insulin concentrations were unaffected ($P = 0.81$; Table 1) by central infusion of BHB; however, insulin concentrations were lower ($P < 0.01$) in RES ewes. In agreement, Zarrin et al. (2013) reported unchanged insulin concentration in cows intravenously infused with BHB. Likewise, serum insulin concentrations were not different between control and centrally infused BHB rats (Park et al., 2011). The increased nutrient intake in MAINT ewes suggests the increased insulin may be attributable to augmentation of insulin mediated peripheral glucose disposal (Schugar et al., 2012). In ruminants, ketone bodies represent a modest stimulus for insulin secretion, which may explain the unchanged insulin concentration in BHB infused ewes (Jordan and Phillips, 1978). The decrease in insulin concentration in RES ewes is further explained by insulin being directly related to feed and energy intake (Brockman and Laarveld, 1986).

Endogenous BHB concentration was unaffected ($P = 0.73$; Table 1) by exogenous BHB infusion. In addition, dietary restriction did not influence ($P = 0.86$) circulating endogenous BHB concentration. According to Carneiro et al. (2016), short-term infusion of BHB into rat brains resulted in dysregulation of metabolic normalization; however long-term infusion resulted in a counter-regulatory response of metabolic normalization. Additionally, the lack of elevated BHB in RES ewes that were in a NEB state may indicate a more metabolically adaptive response than MAINT ewes (Mulliniks et al., 2016). This speculation may be further explained by heterostatsis, or long-term adaption to a new steady state (Selye, 1973). Ketone bodies are utilized as alternate fuel sources during times of physiological stress or starvation, allowing for a glucose-sparing effect in certain tissues (Zarrin et al., 2013). While ketone bodies are always present in circulation, increases in ketone bodies are frequently observed concurrently with a NEB and lack of glucose (Laffel, 1999).

Serum NEFA concentration increased ($P < 0.01$; Table 1) with RES ewes; however, NEFA concentration was unaffected ($P = 0.74$) by BHB infusion. Elevated NEFA concentrations have been previously reported in feed restricted steers (Hayden et al. 1993; Wertz-Lutz et al., 2008), thus indicating a prolonged catabolic state. The increase in NEFA in RES ewes was expected due to an expected increase in tissue mobilization from the feed restriction. In agreement with our results, decreased insulin concentrations have been reported with elevated NEFA concentrations as a result of increased lipid mobilization (Fiore et al., 2014).

Circulating SUN was unaffected ($P = 0.88$; Table 1) by infusion of BHB. Similarly, SUN was not different between lactating beef cows with low or elevated BHB concentrations (Mulliniks et al., 2013). Though SUN concentrations were unaffected by infusion of BHB, SUN concentrations increased ($P < 0.01$) in RES ewes. Fenwick et al. (2008) reported elevated SUN concentrations in dairy cows experiencing severe NEB, which may indicate an increased lean protein mobilization and explain our increase in SUN in RES ewes. Increased catabolism of amino acids from tissue proteins is stimulated by energy deficient states which can result in elevated urea N concentrations (Bell, 1995).

Concentration of BHB in the CSF was unaffected ($P = 0.49$) by diet. Likewise, CSF BHB concentration was unaffected ($P = 0.86$) by exogenous administration of BHB. It has been previously reported an increase uptake of ketones by the brain during prolong periods of starvation (Gjedde and Crone, 1975); thus, indicating the potential for BHB to act as negative energy signal. Additionally, the rate of ketone body utilization by the brain is a direct reflection of circulating plasma levels (Hasselbalch et al., 1995). Kammula (1976) reported the ovine brain oxidizes significant amounts of ketones bodies irrespective of nutritional state. Additionally, it has been reported an increased utilization and uptake of ketones by the brain during times of hyperketonemia (Kammula 1976). Furthermore, according to Titgemeyer et al. (2011) a G-protein, GPR109A, may allow the central nervous system to monitor circulating levels of BHB concentrations. Therefore, BHB may potentially affect intracellular signaling through transport or by binding to the membrane of GPR109A (Laeger et al., 2012).

IMPLICATIONS

Due to the decrease in circulating glucose concentrations exhibited in beta-hydroxybutyrate infused ewes in this study, beta-hydroxybutyrate may serve as a potential energy signal to modulate peripheral metabolic status through a glucose-sparing effect. Whole animal metabolism is coordinated by glucose through the orchestration of alterations both centrally and peripherally. Additionally, chronic feed restriction may further comprise overall metabolic state; however, central infusion of beta-hydroxybutyrate in the lateral ventricle did not further exacerbate the effect of negative energy balance.

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Umbilical blood flow and conceptus measurements in single and twin pregnancies in ewes

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ABSTRACT: Twin calves and lambs are usually born lighter than their single counterparts. Similarly, decreased lamb birth weights have been repeatedly associated with decreased uterine and umbilical blood flows. In this study, we investigated if birthweights in twins are associated with umbilical blood flow (UBF). Singleton (n = 6) and twin (n = 7) carrying ewes were randomly selected and assessed throughout pregnancy. Ewe BW was measured every 10 days from d 20 until d 130 of gestation. Beginning on d 50, and every 10 days until d 110, conceptus measurements and umbilical hemodynamics were obtained via Doppler ultrasonography. Twin carrying ewes tended (P = 0.07; unprotected F test) to be heavier than singleton carrying ewes by d 130. There was a fetal number × day interaction (P = 0.05) for fetal abdominal girth, with singletons tending (P = 0.07) to be larger than twins on d 80 of gestation. Twin fetuses had greater biparietal distance by d 110 (P = 0.04; unprotected F test). Ewe BW as well as all fetal dimensions increased (P < 0.01) with day of gestation. There was no fetal number × day interaction nor main effect of fetal number ($P \geq 0.12$) for UBF or resistance index. Umbilical blood flow was greater (P = 0.04; unprotected F test) in twins on d 50 of gestation. A fetal number × day interaction (P = 0.03) was observed for pulsatility index, with twins tending to be greater ($P \leq 0.06$) than singletons on d 100 and 110. Placentome size tended to have a fetal number by day interaction (P = 0.07) and showed a main effect of fetal number (P < 0.01). Single and twin placentas had similar (P = 0.19) placentome size on d 50. However, on d 60, 70, 80, 90, 100 and 110 twins had larger ($P \leq 0.05$) placentomes than singletons. Average lamb birth weights and total cotyledon number were similar ($P \geq 0.40$) between treatments and when totaled, cotyledon and fetal membrane weights were greater (P < 0.01) in twins vs. singletons. Perhaps the similar UBF, placental growth, and placental weights per fetal unit allowed the twins in this study to have a similar birth weight as the singletons.

Key words: Placenta, Sheep, Umbilical blood flow
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INTRODUCTION

Twin calves and lambs are usually born lighter than their single counterparts (Dwyer et al., 2005; Echternkamp et al., 2006). In sheep, total placentome or cotyledonary number and weight are increased in twin versus singleton pregnancies, although the average weight and number are reduced (Alexander, 1964; Vatnick et al., 1991; Vonnahme et al., 2008). In cows, reduced placental perfusion causes a reduction in fetal growth (Ferrell and Reynolds, 1991). Similarly, decreased lamb birth weights have been repeatedly associated with decreased uterine and umbilical blood flows (Dwyer et al., 2005; Lemley et al., 2012). Doppler ultrasonography (US) has been successfully used to assess umbilical artery and vein hemodynamics (Galan et al., 1998; Rigano et al. 2001; Ferrazzi et al., 2002; Lemley et al., 2012). To our knowledge there are few data investigating hemodynamic differences between single and twin pregnancies in mammals. In humans, fetal aortic isthmus pulsatility index (PI; negatively correlated with placental flow) is similar between healthy single and twin babies (Gámez et al., 2015). In sheep, a study reported no difference in umbilical blood flow (UBF) between single and twin pregnancies (Erdogan et al., 2016). The same study reported random variations in PI, peak systolic and end diastolic velocities in the umbilical cord as well as in biparietal and thoracic fetal distances (Erdogan et al., 2016). However, the mentioned

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experiment did not report placentome sizes or any lambing data that could be related with their US observations (Erdogan et al., 2016). In this study, we investigated if birthweights in twins are associated with UBF. We also investigated fetal and placentome sizes in twins and singletons throughout gestation and related the US data with birth and placental weights. We hypothesized that lighter birthweights in twins would be related to a decreased UBF of twins vs. singletons. We also hypothesized that the total placenta from twin pregnancies would have greater mass with more cotyledons than singleton pregnancies.

MATERIALS AND METHODS

Animals and experimental design

Animal care and use were according to protocols approved by the North Dakota State University Animal Care and Use Committee (IACUC #A15076). Multiparous Dorset ewes (n = 42) were randomly selected from the NDSU Sheep Unit. Estrus was synchronized using progesterone containing Controlled Internal Drug Release (CIDR) devices. After synchronization, ewes were transported to the NDSU Animal Nutrition and Physiology Center (ANPC). At ANPC ewes were housed in a common pen and were fed a diet of pellets and hay for ad libitum intake that met or exceeded NRC recommendations. This feeding protocol was maintained throughout the duration of the experiment. All ewes were bred to one ram and breeding dates were recorded. Pregnancy diagnosis and fetal enumeration was performed on d 30 of gestation via US (Aloka Prosound Alpha 6). Six singleton and seven twin carrying ewes were randomly selected and assessed throughout pregnancy. Ewe BW was measured every 10 days from d 20 until d 130 of gestation. After d 130 of gestation, ewes were transported back to the NDSU Sheep Unit and received hay and water for ad libitum intake. Birth was monitored and placentas were collected. Lamb birth weights, placental weights, and number of cotyledons were recorded.

Gestational measurements

Beginning on d 50, and every 10 days until d 110, ewes were restrained so that conceptus measurements and umbilical hemodynamics could be obtained. All measurements were obtained before 10 AM. Conceptus measurements included the length and width from 10 random placentomes, fetal biparietal and abdominal lengths, and kidney length and width in duplicate every 10 days. Placentome area was calculated by multiplying width by height measurements for each placentome. For umbilical hemodynamic measurements, Doppler mode was used to obtain UBF, PI and resistance index (RI) as previously described (Lemley et al., 2012).

Statistical analyses

The research was conducted as a completely randomized design with repeated measures. Repeated data were analyzed using the MIXED procedure of SAS (SAS software version 9.4, SAS Institute, Cary, NC). Ewe was treated as a random independent variable; fetal number and day were treated as fixed effects. Placentome area, UBF, PI, RI, fetal biparietal and abdominal lengths, kidney length and width and ewe body weight were the dependent variables. Least square means were separated using the PDIFF option of the LSMEANS statement. Birth data were analyzed using the GLM procedure. Means were separated using the LSMEANS statement. P values ≤ 0.05 are considered significant. Tendencies are described when P values are > 0.05 but ≤ 0.10.

RESULTS

On d 20 of gestation, ewes had similar weights ($P = 0.78$; 68.08 ± 3.08 kg; Figure 1). There was no fetal number × day interaction nor main effect of fetal number on ewe weights throughout the experiment ($P > 0.34$). However, twin carrying ewes tended ($P = 0.07$; unprotected F test) to be heavier than singleton carrying ewes by d 130 (Figure 1; 76.58 vs. 68.19 ± 3.27 kg). There was a fetal number × day interaction ($P = 0.05$) for fetal abdominal girth, with singletons tending ($P = 0.07$) to be bigger than twins on d 80 of gestation (5.00 vs. 4.78 ± 0.08 cm). The remaining days of gestation fetal girth was not different ($P \geq 0.11$). Fetal biparietal distance had no fetal number by day interaction nor main effect of fetal number ($P \geq 0.18$). However, an unprotected F test revealed a greater ($P = 0.04$) biparietal distance in twin vs singleton fetuses by d 110 of gestation (5.52 vs. 4.93 ± 0.19 cm). There was no fetal number × day interaction nor main effect of fetal number ($P \geq 0.26$) for kidney length and width throughout the experiment (data not shown). Ewe BW as well as all fetal dimensions showed a significant effect of day ($P < 0.01$), with all measurements increasing with the advancement of gestation.

There was no fetal number × day interaction nor main effect of fetal number ($P \geq 0.12$) for UBF or RI. Nevertheless, an unprotected F test revealed that UBF was greater ($P = 0.04$) on d 50 of gestation in twins vs. singletons (27.37 vs. 20.95 ± 1.99 ml/min). Pulsatility index showed a fetal number × day interaction ($P = 0.03$), with twins tending to be greater ($P \geq$

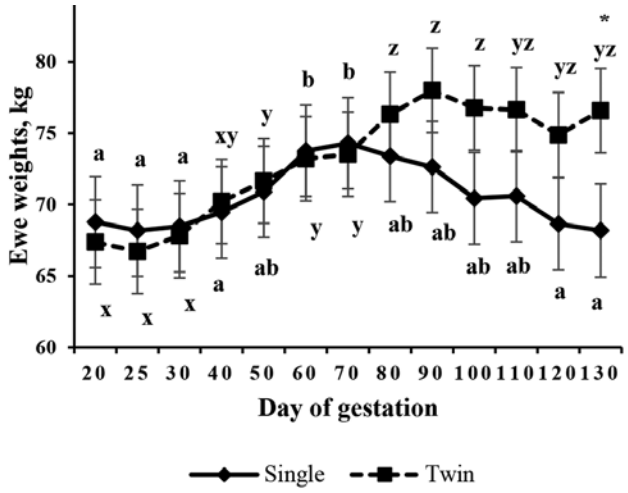

Figure 1. Fetal number effect on ewe weight from d 20 to 130 of gestation. Singles vs. twins. [ab]LSMeans ± SEM within Single differ $P < 0.05$; [xyz]LSMeans ± SEM within Twin differ $P < 0.05$. Differences between Single and Twin are denoted by *$P \leq 0.10$.

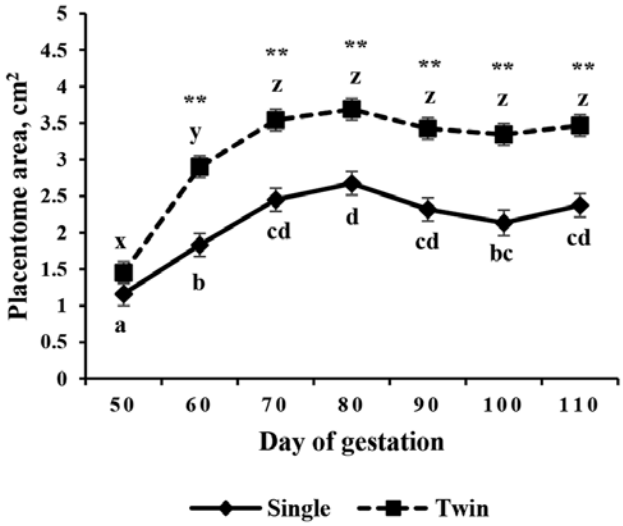

Figure 3. Fetal number effect on placentome size from d 50 to 110 of gestation. Singles vs. twins. [abcd]LSMeans ± SEM within Single differ $P < 0.05$; [xyz]LSMeans ± SEM within Twin differ $P < 0.05$. Differences between Single and Twin are denoted by **$P \leq 0.05$.

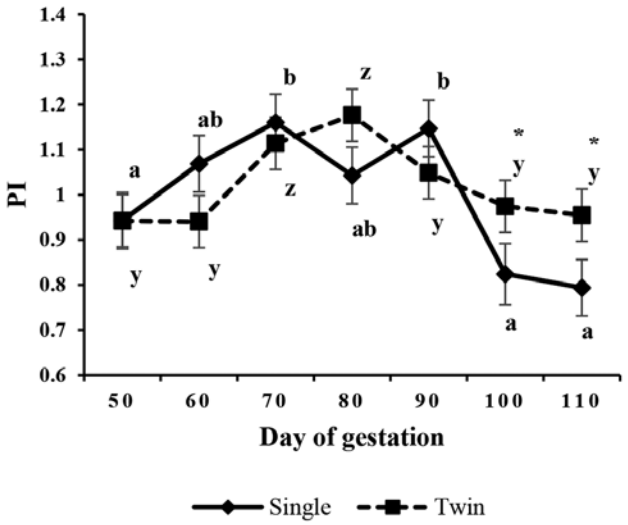

Figure 2. Fetal number effect on umbilical artery PI from d 50 to 110 of gestation. Singles vs. twins. [ab]LSMeans ± SEM within Single differ $P < 0.05$; [yz]LSMeans ± SEM within Twin differ $P < 0.05$. Differences between Single and Twin are denoted by *$P \leq 0.10$.

0.06) than singletons on days 100 and 110 (Figure 2). All umbilical hemodynamic measurements had a significant effect of day ($P < 0.01$), with UBF increasing as gestation advanced and PI and RI varying independently with day of gestation (Figure 2). Placentome size tended to have a fetal number × day interaction ($P = 0.07$) and showed a main effect of fetal number ($P < 0.01$). Single and twin placentas had similar ($P = 0.19$) placentome size on d 50 (Figure 3). However, on d 60, 70, 80, 90, 100 and 110 twins had larger ($P \leq 0.05$) placentomes than singletons (Figure 3).

Average lamb birth weights and total cotyledon number were similar ($P \geq 0.40$) between treatments

TABLE 1. Offspring and placental lambing data[1]

	Singles	Twins	SEM	*P*
Average birth weight, kg	4.25	4.38	0.64	0.88
Total cotyledon number	96.8	104.2	5.9	0.40
Total placental weight, g	310.7	738.1	56.9	< 0.01
Total cotyledon weight, g	80.9	199.6	21.9	< 0.01
Total fetal mem. weight, g	203.9	493.9	33.2	< 0.01

[1]$P \leq 0.05$ are considered significant; mem. = membrane.

(Table 1). However, total placental, cotyledon and fetal membrane weights were greater ($P < 0.01$) in twins vs. singletons (Table 1).

DISCUSSION

We hypothesized that decreased birth weights in twin lambs would be related to decreased UBFs. However, our results did not show decreased birthweights in twin lambs. Similarly, twin lambs did not have decreased average placental weights or cotyledon weights (data not shown). Interestingly, UBF was also not different between single and twin fetuses. In cows, a study showed decreased UBF in twin calves (Ferrell and Reynolds, 1991). Unfortunately, fetal weights were not reported (Ferrell and Reynolds, 1991). Decreased UBFs are related with decreased fetal and lamb birth weights (Dwyer et al., 2005; Lemley et al., 2012). Similarly, birth weight and weight of the complete placenta are often positively correlated in man, pig, guinea pig, rabbit and sheep (Alexander, 1964; Vatnick et al., 1991; Vonnahme and Ford, 2004; Dwyer et al., 2005). In sheep, positive correlations have also been

reported between birth weights and cotyledonary weights (Alexander, 1964; Dwyer et al., 2005). In our study, increased cotyledon sizes along with similar total cotyledon numbers, average cotyledon weights, UBF and birth weights suggest an effective physiological compensation of the twin placenta. Therefore, similar UBF as well as similar average placental and cotyledonary weights appear to be associated with similar birth weights in twin vs. singleton lambs.

Twin bearing ewes showed a consistent increase in weight after d 70 of gestation when compared to singletons. While we did not monitor intake or total gravid uterine weights, this increase could partially be explained by the increase in placentome mass and greater fetal mass that was being carried in twin vs. single pregnancies. While placentome sizes would have peaked between d 80 and 90, the majority of fetal weight gain happens after d 90 (Redmer et al., 2004).

The fact that abdominal girth was greater for singleton fetuses on d 80 of gestation and fetal biparietal distance was greater for twin fetuses on d 110 of gestation could mean that twin fetuses were smaller than singleton fetuses earlier on gestation and that twin fetuses recovered and surpassed singleton size during later gestation. Similarly, this could be hypothesized as a consequence of an increase in placentome size in twins vs. singles during the second half of gestation. However, the lack of other fetal measurements (kidney size) that concur with these data as well as more solid statistical values for these findings restrain us from making such assumptions.

IMPLICATIONS

Twins are commonly lighter than singleton lambs. Average UBF, placental and cotyledonary weights are also commonly decreased in twins. Our findings reaffirm the importance of adequate placental development and UBF in order to maintain adequate fetal growth. Similar UBF, placental growth and placental weights per fetal unit seem necessary in twins in order to attain similar birthweights as singletons.

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Using corn supplementation for overwintered beef cows during mid- to late-gestation: Uterine hemodynamics, placental vascularity, and neonatal performance

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ABSTRACT: Improper maternal nutrition during pregnancy can lead to poor carcass quality in offspring. Gestating beef cows fed low-quality forage diets in extensively managed operations are often at risk of nutrient restriction. Thus, the objective of this study was to evaluate the effects of supplementing low-quality forage with corn to gestating beef cows by tracking uterine hemodynamics and neonatal performance. We hypothesized that mid- to late-gestating beef cows receiving corn supplementation would have greater uterine hemodynamics, increased placental vascularity, and give birth to faster growing calves. Forty-seven multiparous Angus beef cows carrying bull calves were assigned randomly to treatments receiving corn supplementation at 0.2% of BW (SUP; n = 24) or no supplement (CON; n = 23). All cows were fed the same basal diet (60% hay, 30% wheat straw, and 10% concentrated separator by-product: CSB). Intake was monitored individually with Insentec feeders from d 100 of gestation through calving. Uterine hemodynamics were monitored using Doppler ultrasonography every 28 d from the start of supplementation until d 240 of pregnancy. At birth, pair weights, colostrum samples, and placental tissues were collected. The calves were again weighed at 3 wk postpartum and weaning (d 168). All measurements and data collected were analyzed with the MIXED procedure of SAS and means were separated using the LSMEANS statement. Corn supplementation reduced roughage intake ($P < 0.001$) in SUP cows; however, kg of TDN consumed per day tended to be ($P = 0.06$) increased in SUP cows. A day by treatment interaction was observed for cow BW ($P < 0.001$) and BCS ($P = 0.03$) with SUP cows gaining more BW and BCS and having increased ADG ($P < 0.001$). Uterine hemodynamics ($P > 0.20$), colostrum production ($P = 0.64$), and placental weight measurements ($P > 0.20$) were not altered by maternal corn supplementation. However, placental vascular surface density was suppressed ($P < 0.01$) by maternal corn supplementation. While calf birth weights were not altered by maternal corn supplementation ($P > 0.50$), calves from SUP dams were heavier at 3 weeks postpartum ($P = 0.05$) but not at weaning ($P > 0.6$). While corn did decrease maternal roughage intake, it appears to be a good substitute for hay as it does not have negative effects on uterine blood flow or calf growth. Depending on the cost of feed inputs, this feeding strategy could be economically advantageous to the producer.

Key words: Beef cow, fetal programming, placenta, uterine blood flow
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INTRODUCTION

Inadequate maternal nutrition during pregnancy can lead to a decrease in carcass quality of the offspring, including altered fat deposition, muscle fiber type and reduced meat quality (Wu et al., 2006). In fact, offspring of dams protein restricted during late-pregnancy had reduced feedlot performance and intramuscular fat accretion (Stalker et al., 2006; Funston et al., 2010). Unfortunately, nutrient restriction is still common in extensively managed, overwintered, ges-

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TABLE 1. Analyzed composition of cow diets derived from hay, straw, and concentrated separator by-product (CSB) during gestation, and straw, silage, and dried distiller's grains (DDGS) during early lactation

	Ingredient, %					Nutrient[1], %					
Item	Hay	Straw	Concentrated separator by-product	Silage	DDGS	Ash	CP	NDF	ADF	Ca	P
Gestation diets											
d 100-153	45	45	10	—	—	11.9	6.4	67	41.3	0.28	0.12
d 154-0265	60	30	10	—	—	11.6	7.1	64.5	38.9	0.37	0.26
Corn[1]						1.5	7.6	14.1	3.2	0.01	0.54
Lactation diet d 266 -3 w											
Lactation	—	45	—	30	25	11.2	11.6	61.9	36.1	0.48	0.37

[1]Nutrient analysis of TMR of each diet fed during gestation and lactation.

[2]SUP cows received corn at 0.2% body weight, CON animals received no corn.

tating beef cows due to limited access to high quality forage. Nutrient restriction can be resolved by improving the forage quality of cows during late gestation, but if that feed resource is not available, an alternate feeding strategy should be employed.

One way to estimate nutrient supply to the gravid uterus is by measuring uterine arterial blood flow (Ferrell, 1991). Doppler ultrasound is a non-invasive, repeatable way to measure changes in uterine hemodynamics to detect the consequences of poor maternal nutrition and target when therapeutic interventions should be applied (Vonnahme and Lemley, 2011). We previously demonstrated that the supplementation of dried distiller's grains plus solubles (**DDGS**) with ad libitum access to low-quality forage to beef cows in late gestation increased dry matter intake and uterine blood flow to the gravid uterus thereby improving calf birth weights (Kennedy et al., 2016b). Thus, it is logical to target winter nutritional management strategies during gestation to increase uterine blood flow for improved fetal development and carcass characteristics.

We hypothesized that corn supplementation of beef cows during mid-to late-gestation would result in increased feed intake, greater total uterine blood flow, and birth heavier, faster growing calves. The objectives of this study were to evaluate the effects of corn supplementation in gestating beef cow on feed intake, uterine hemodynamics, placental morphology, colostrum production, and neonatal performance and growth.

MATERIALS AND METHODS

Experimental design, cows, and dietary treatments: All procedures were approved by the North Dakota State University Animal Care and Use Committee (IACUC #A16010). Forty-seven multiparous beef cows (predominately Angus breeding) carrying bull calves (confirmed via ultrasonography at d 70 of gestation) from the Central Grasslands Research and Extension Center in Streeter, ND were transported to the Beef Cattle Research Complex in Fargo, ND. The cows were assigned randomly to treatments (**CON**; n = 23) receiving ad libitum access to a low-quality forage-based diet only (57.54% TDN, 6.4% CP) or (**SUP;** n = 24) an additional corn supplement at 0.2% BW (94.5% TDN, 7.64% CP with ad libitum access to low-quality forage to increase BCS. The basal forage diet (Table 1) was provided ad libitum to all cows for the duration of the experiment, but corn supplementation was provided to SUP cows at a limited amount (the system rejected the animal after consuming 0.02% BW). The cows were stratified by BW and BCS across treatments. Cows weighed 661 ± 7.8 kg, had a BCS of 5.2 ± 0.1 (9-point scale), and were 7.5 ± 0.2 years old at the start of the study. The cows were housed in 4 adjacent drylot pens (12 or 13 cows per pen). After a 3 wk acclimation and training period, dietary treatments were applied and intake was monitored and controlled via roughage intake control feeders (Insentec B.V., Markenesse, The Netherlands) beginning on d 100 of gestation for 22 wk. At d 265 of pregnancy, all cattle were fed a common lactation diet (45 % straw, 30% corn silage, and 25% DDGS) that was higher in protein (11.6% CP) until 3 wk postpartum. Basal forage diet during both gestation and lactation were sampled weekly and analyzed for nutritional content (Table 1) using the methods reported by Kennedy et al. (2016a). All cows had free access to water and a trace-mineralized salt block throughout the experiment.

Uterine hemodynamics & parturition: Uterine hemodynamic measurements were taken in accordance with Kennedy et al. (2016b). Briefly, Doppler ultrasonographic measurements were measured from both uterine arteries at d 100, 125, 150, 210, and 240

of pregnancy. A minimum of 6 waveforms were assessed. All cows were weighed and body condition scored using a 1 to 9 scale (1 = emaciated and 9 = obese) at each ultrasound time point.

Calving was monitored continuously on a 24 h basis from d 265 of gestation until all calves were born. Immediately after calving, the pairs were brought into the barn for monitoring and health analysis. Placental, back right quarter-colostrum, and calf measurements at birth followed the procedures as described (Kennedy et al., 2016b). After weighing, representative cotyledons were processed and fixed in formalin for histological preparation.

Placental vascularity: Fixed cotyledons were embedded in paraffin and sectioned on a microtome at 5 μm. Sections were stained with hematoxylin & eosin. Four representative images were taken of each sample at 20x magnification with a Axio Imager.M2 microscope equipped with an AXioCamHR3 color camera and AxioVision 4.8 software (Carl Zeiss International, Jena, Germany). Image analysis was performed using the Image-Pro Premier 9.2 software package (Media Cybernetics, Inc., Rockville, MD). At least three randomly chosen polygons were drawn in each image to determine vascular area density (**VAD,** % = vascular area/unit tissue area) and vascular surface densities (**VSD**, μm^2/unit tissue volume in μm^3) as described by Borowicz et al. (2007). Total vascular volume in ml was calculated as VAD × total cotyledonary weight (g), assuming that VAD is equivalent to vascular volume density (Borowicz et al., 2007).

Calf growth: Calf BW was determined at 24 h and 3 wk postpartum and at weaning (168 ± 16 days postpartum).

Statistical analysis: All measurements and data collected were analyzed with generalized least squares mixed procedure of SAS (SAS Institute, Cary, NC) with repeated measures using least squares. Model statements included cow, maternal diet (CON vs. SUP), day of gestation, and a diet by day of gestation interaction. Sire was treated as a random variable. All meaningful interactions were considered and in the absence of interactions, main effects were discussed. Various covariance structures were assessed; those structures with the lowest fit statistics were utilized (Wang and Goonewardene, 2004). When appropriate, p-values for differences of means were determined with PDIFF option of LSMEANS statement.

RESULTS

Cow diets, BW, and BCS: Corn supplementation reduced roughage intake ($P < 0.001$) in SUP cows (12.8 vs. 14.4 ± 0.29 kg/d DM basis), however, kg of TDN consumed tended to be greater in SUP cows ($P = 0.06$) because of the energetically dense corn (1.47 ± 0.1 kg/d DM basis) that substituted for the additional roughage intake in SUP cow diets. Crude protein consumed was not different ($P = 0.81$) between dietary treatments (0.99 vs. 0.99 ± 0.2 kg/d). Additionally, day by treatment interaction were observed for cow BW ($P < 0.01$) and BCS (Figure 1; $P \leq 0.05$) as SUP cows gained more BW and BCS across gestation. Additionally, supplemented cows also had greater ADG ($P < 0.001$) than CON cows (0.68 vs 0.46 ± 0.08 kg/d).

Blood flow and resistance indices: No day by treatment interaction (P =0.27) or treatment effect ($P \geq 0.47$) was observed for ipsilateral (8.38 vs. 9.38 ± 1.62 L/min) or contralateral (4.24 vs. 3.73 ± 0.41 L/min) uterine arterial blood flow (Figure 1). No day by treatment interaction ($P = 0.98$) or dietary treatment effect ($P = 0.65$) was observed for total uterine blood flow (Figure 1; 12.37 vs. 12.79 ± 0.77 L/min). As expected, both ipsilateral and contralateral uterine blood flow increased as gestation advanced ($P < 0.01$) in both treatment groups. Furthermore, pulsatility index (**PI**), a commonly utilized index to indicate tissue perfusion (Ginther, 2007), was also not altered by corn supplementation as no day by treatment interaction *(P* = 0.67) or treatment effect ($P = 0.15$) was observed on the side ipsilateral (1.26 vs. 1.20 ± 0.04) to the conceptus, although PI decreased during gestation ($P < 0.01$). Similarly, there was no day by treatment interaction ($P = 0.16$) or treatment effect ($P = 0.51$) detected for PI contralateral (1.31 vs 1.34 ± 0.07) to the developing fetus, even though PI decreased over gestation ($P < 0.01$). For resistance index (**RI**), an indicator of arterial resistance (Ginther, 2007), no day by treatment interaction ($P = 0.55$) was observed. However, RI tended to be altered by maternal dietary treatment as corn supplementation increased ($P = 0.06$) vascular resistance on the side ipsilateral to the conceptus (0.67 vs. 0.65 ± 0.01) while RI for both treatments decreased over gestation ($P < 0.01$). Maternal diet did not influence contralateral RI as no day by treatment interaction ($P = 0.13$) or dietary treatment effect ($P = 0.26$) was detected (1.31 vs. 1.34 ± 0.07) but contralateral RI still decreased ($P < 0.01$) over time in both treatments. Additionally, for maternal heart rate no day by treatment interaction ($P = 0.88$) or maternal dietary treatment effect ($P = 0.27$) was detected over gestation (68.6 vs. 71.3 ± 3.7 bpm) although heart rate increased over gestation ($P < 0.01$).

Parturition: At parturition, dam BW ($P > 0.50$; 713.5 vs. 702 ± 13.4 kg) and BCS ($P > 0.20$; 5.6 vs. 5.6 ± 0.1) were similar for CON and SUP, respectively. Calf birth weights (39.8 vs. 39.6 ± 1.0 kg) were also unaffected ($P > 0.50$) by maternal corn supplementation with calves from SUP dams being similar

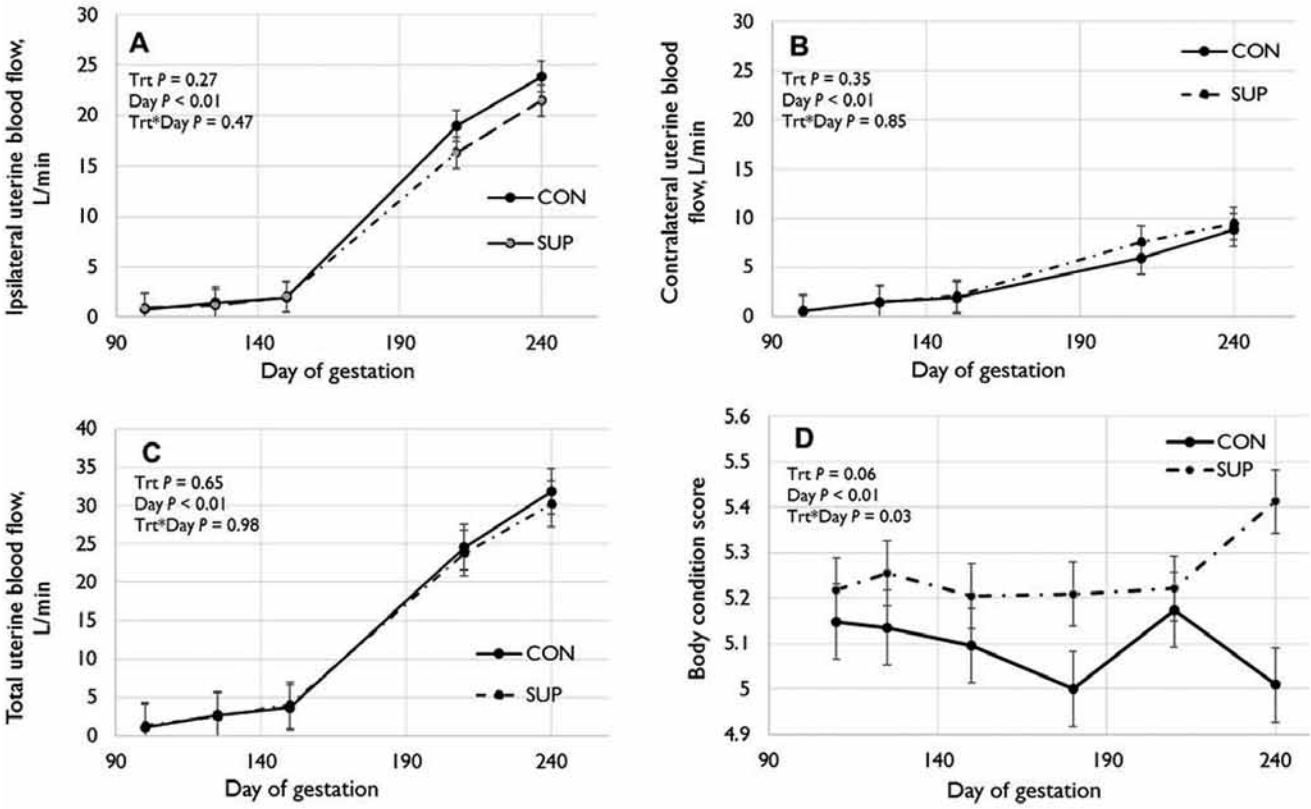

Figure 1. Ipsilateral uterine arterial blood flow (A) and contralateral uterine arterial blood flow (B) and total uterine arterial blood flow (C) and body condition scores (D) of beef cows fed the CON or SUP diets from d 100 to d 240 of gestation.

to calves from CON cows at birth. Additionally, calf heart girth ($P > 0.40$; 80.1 vs. 80.9 ± 0.77 cm) and crown-rump length ($P > 0.20$; 86.9 vs. 84.0 ± 4.32 cm) at birth were also not influenced by maternal diet. Interestingly, the SUP cows tended ($P = 0.07$) to have shorter gestation lengths (277.7 vs. 279.6 ± 1 day) with no difference in incidence of dystocia ($P > 0.5$) compared to CON cows. Corn supplementation also did not negatively impact on colostrum production ($P = 0.64$; 633 vs. 707 ± 110 g) at birth. Placental measures collected at birth were similar across treatments ($P > 0.20$) for total placental weights (4251.1 vs. 4462.9 ± 275 g), total cotyledon number (80.0 vs. 90.8 ± 17.8) and total cotyledonary weight (1603.5 vs. 1734.9 ± 126.3 g). Furthermore, placental efficiency (fetal: placental weight) did not differ because of maternal corn supplementation ($P > 0.30$). No differences ($P > 0.50$) in intercotyledonary weight (2141.5 vs. 2258.3 ± 159 g) or largest cotyledonary weight (83.3 vs. 85.4 ± 7 g) were found. However, maternal diet did alter the size of the smallest cotyledon weight with SUP cows having a larger ($P = 0.04$) smallest cotyledonary weight than CON cows (0.90 vs. 0.39 ± 0.4 g).

Placental vascularity: Interestingly, SUP cows tended to have decreased ($P = 0.10$) cotyledonary VAD (10.77 vs. 8.90 ± 1.01%) compared to CON cows. Additionally, SUP cows had ($P < 0.01$) decreased cotyledonary VSD (1024.0 vs. 626.8 ± 97 $\mu m^2/\mu m^3$) compared to CON cows. However, total vascular volume was not altered by dietary treatment ($P > 0.20$; 190.3 vs. 165.6 ± 24.3, mL).

Calf growth: Similar to measurements at parturition, 24 h calf BW were also not altered ($P = 0.68$) by maternal diet (40.0 vs. 40.5 ± 1.8 kg) with calves from corn fed dams weighing similarly to offspring from hay fed cows. At 3 weeks post-partum, calves from SUP dams were heavier ($P = 0.05$) than calves from CON dams (72.5 vs 68.9 ± 3.5 kg). However, this effect disappeared by weaning as the offspring from SUP and CON dams were similar in weight ($P = 0.64$; 291.5 vs. 281.4 ± 8.1 kg, respectively).

DISCUSSION

We reject our hypothesis that corn supplementation from mid- to late-gestation would increase uterine blood flow to the gravid uterus and increase calf birth weight. While TDN intake was increased in SUP cows, this did not alter uterine blood flow. When cows were nutrient restricted to 60% of their daily NRC requirements during early (d 30 to 140) pregnancy, uterine blood flow was unchanged (Camacho et al., 2014).

However, when multiparous cows during late gestation (d 180 to 246) were provided ad libitum access to forage and limit fed a DDGS protein supplement (0.3% BW), dry matter intake was increased (Kennedy et al., 2016a) and uterine arterial blood flows and calf birth weights were both increased (Kennedy et al., 2016b). Perhaps metabolizable protein is driving these changes in uterine blood flow rather than total energy intake. Not only does protein supplementation positively affect birth weight, but other studies report that maternal dietary protein amount during gestation affects calf growth and subsequent carcass quality. For example, Shoup et al. (2015) examined the effects of maternal dietary protein supplementation on offspring carcass quality and found that dams receiving additional protein during late gestation produced calves with increased marbling and backfat at slaughter. However, it also appears that protein supplementation alone is not solely driving blood flow and increased fetal growth. When beef cows were limit-fed forage while being fed a DDGS supplement in late gestation (d 190 to d 240), a decrease in uterine blood flows occurred in supplemented cows (Mordhorst et al., 2016). Surprisingly, calf birth weight was unaffected despite the decrease in uterine blood flow (Mordhorst et al., 2016). In the current study, unlike the DDGS supplementation which had a positive impact on forage intake and fetal growth, the energy (corn) supplementation decreased roughage intake and did not alter fetal growth. Although the dams had ad libitum access to their basal diets during mid- to late-gestation, the corn supplement had negative impacts on roughage intake, but not TDN intake. Additionally, although most measurements of uterine blood flow and placental morphology were unaltered by corn supplementation, placental vascularity was suppressed in the cotyledons from SUP dams. Perhaps this is because the fetuses from cows supplemented with corn were already receiving the necessary nutrients for normal fetal growth, and did not need to produce as many angiogenic factors that could alter or increase cotyledonary vasculature. This potential energy abundance is supported by the increased ADG and greater daily TDN consumption of SUP cows. Interestingly, in a study where beef cows were nutrient restricted (60% of NRC) in early pregnancy (until d 120) but later realimented, decreased cotyledonary VAD and VSD were observed at d 250 when compared to CON (100% of NRC) cows (Vonnahme et al., 2007). In spite of mixed vascularity results in response to maternal diet, it is important to note that in this study, the reduction in cotyledonary vascularity of the SUP group was not reflected by decreased vascular volume and did not impede normal fetal or calf growth. Regardless of the dietary effects of corn on cotyledonary vascularity, the true effects of maternal corn supplementation on postnatal growth and performance seem to be highly variable. A similar study by Radunz et al. (2012) found that beef cows limit-fed corn (63% of the diet) during late gestation produced calves that were heavier at birth than calves from dams fed hay. Additionally, calves from cows fed corn also had heavier weaning weights, faster glucose disappearance rates, and decreased intramuscular fat at slaughter than calves from hay fed dams (Radunz et al., 2012). Growth and carcass traits for calves from the current project are yet to be assessed.

IMPLICATIONS

While dietary energy intake in cows seems to alter cotyledonary placental capillary vascularity at term, energy supplementation seems to have limited impacts on prenatal growth in the current study. Perhaps, dietary supplemental protein combined with ad libitum access to forage could be more effective in improving fetal growth by increasing uterine arterial blood flow. This suggests that care should be taken to supply dams with adequate protein during gestation to prevent altered hemodynamics and reduced fetal growth. However, because corn supplementation does not affect fetal growth and birth weights, corn appears to be a substitute for hay. In fact, this feeding strategy could be economically advantageous to the producer especially depending on the cost of feed inputs or availability of resources.

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Effects of metabolizable protein supply and fetal number on placental binucleate cells in pregnant sheep during late gestation

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ABSTRACT: We hypothesized that maternal metabolizable protein (MP) levels and fetal number would impact placental binucleate cell (BNC) number, size, and BNC percentage per cotyledon area in sheep. Multiparous Rambouillet pregnant ewes (n = 44) carrying singleton or twin lambs were randomly assigned at d 100 of gestation to one of the three nutritional treatments 60% MP (MP60; n = 15), 80% MP (MP80; n = 15), or 100% MP (MP100; n = 14) of NRC requirements. The resulting arrangement of treatments was a 2 × 3 factorial. Necropsies were performed on day 130 ± 1 of gestation. Placentomes were fixed in formalin and sectioned for immunofluorescent staining. Image analyses were performed to determine BNC number, size percentage BNC per cotyledonary area and percentage cotyledon. There was no MP level × fetal number interaction ($P \geq 0.15$) or main effects ($P \geq 0.21$) for BNC number or the proportion of the cotyledon that was composed of BNCs. There was a MP level × fetal number interaction ($P = 0.04$) for BNC size where MP60 ewes carrying twins had placentomes with larger BNCs compared to all other groups except MP100 singletons, which did not differ ($P = 0.10$). The proportion of the placentome that was occupied by cotyledonary tissue was greater ($P < 0.01$) for ewes carrying twins (58.63 vs 51.94 ± 1.47%). Perhaps the stress of decreased protein intake as well as carrying twins increased the need for factors that are secreted by BNCs.

Key words: Binucleate cells, gestation, metabolizable protein, sheep
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INTRODUCTION

Maternal nutrition is one of the environmental factors impacting the size and functional capacity of the placenta, uteroplacental blood flow, conceptus nutrient availability and the endocrine milieu (Wu et al., 2006; Vonnahme et al., 2013; Lemley et al., 2014). Adequate protein supply plays a key role for maternal maintenance during pregnancy, lactation performance, and rebreeding success (Bond and Wiltbank 1970; Anthony et al., 1986; Drouillard et al., 1991). Binucleate cells (BNC) are formed by the fusion of the chorionic epithelium and uterine luminal epithelium within the placentome to create a feto-maternal syncytium (Wooding 1982; Wooding and Flint, 1994; Wooding et al., 1996). Some of the placental hormones are produced and secreted from BNC. Binucleate cells (or trophoblastic giant cells as it is called in rodents) produce paracrine and endocrine factors such as placental lactogen, progesterone, vascular endothelial growth factor, and placental growth factor (Hu and Cross, 2010). Lekatz et al. (2015) reported that ewes carrying singleton lambs fed from d 100 to 130 of gestation receiving 60% of metabolizable protein (MP) requirements had greater uterine blood flow compared with ewes fed 100% MP. In this study, we tested our hypothesis that maternal MP and fetal number would impact BNC number, BNC size, and BNC percentage per cotyledonary (COT) area in sheep. We hypothesized ewes restricted in MP, or ewes carrying twins, would have greater numbers or sizes of BNCs to overcome the negative impacts on nutrient exchange during late gestation.

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MATERIALS AND METHODS

Animals

Animals care and use was approved by the Institutional Animal Care and Use Committee at North Dakota State University (#A0921). Multiparous Rambouillet ewes were transported from the Hettinger Research Extension Center to the Animal Nutrition and Physiology Center at North Dakota State University. Rambouillet multiparous ewes (n = 44) carrying singletons or twins were assigned randomly on d 100 of gestation to three groups: 60% MP (MP60; n = 15), 80% MP (MP80; n = 15), or 100% MP (MP100; n = 14) NRC (2007) requirements (Table 1).

Sample Collection

Necropsies were performed on d 130 ± 1 of gestation. Immediately following necropsies, the fetus(es) and placentomes were removed and weighed. Placentomes near the umbilicus were fixed in formalin for histologic analysis. The remaining placentomes were separated into caruncular and COT portions and weighed. Placentomes were sectioned at 3 µm and stained with biotinylated Dolichos Biflurus Agglutinin (DBA) lectin, Texas red-avidin and Fluorescein labeled Griffonia (Bandeiraea) Simplifolica lectin 1 (BSL-1). Five representative images were acquired per section at 20× magnification using Zeiss Inverted AxioObserved.Z1 microscope equipped with Axiocam 506 monochrome camera, and ZEN 2 pro software (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). The number, size, and percentage of BNC per COT area and percentage COT area per placentome section were determined using Image-Pro Primer 9.1 software (Media Cybernetics; Rockville, Maryland, USA).

Statistical Analysis

Ewes carried singletons (n = 28; MP60 = 9, MP80 = 12, MP100 = 7) and twins (n = 16; MP60 = 6, MP80 = 3, MP100= 7). Data were analyzed for fetal number, MP level, and their interaction using the mixed procedure of SAS (SAS Inst. Inc, Cary, NC, USA). Means were separated using the LSMEANS procedure and *P* values ≤ 0.05 were considered different.

RESULTS AND DISCUSSION

Anatomical traits

Placental and fetal measurements have been previously reported (Swanson et al., 2017). Briefly, while diet did not impact fetal or placental measurements, twins had a 1.7-fold increase in fetal membrane weight, 1.6-fold increase in COT, and a 1.4-fold increase in caruncular weights (Swanson et al. 2017). It has been demonstrated by many that the total placental mass in twin-carrying ewes is greater than singletons (Vonnahme et al., 2008).

TABLE 1. Ingredient and nutrient composition of dietary supplements fed to ewes[1]

	Treatment		
Item	MP100	MP80	MP60
Ingredients (% DM)			
Corn	5.00	15.00	18.50
DDGS	30.00	20.00	7.00
Soyhulls			9.50
Nutrient composition			
DM (%)	95.90	95.89	95.51
NEm (Mcal/Kg)	2.14	2.22	2.00
CP (% of DM)	25.03	20.53	13.45
MP (% of DM)	16.31	13.01	8.41
NDF (% of DM)	40.79	32.11	33.61
ADF (% of DM)	11.61	8.33	15.71
Ash (% of DM)	4.38	3.50	3.17

[1]ADF, acid detergent fiber; CP, crude protein; DDGS, dried distillers grains plus solubles; DM, dry matter; MP, metabolizable protein; NDF, neutral detergent fiber; NEm, Net energy for maintenance. Treatment: MP100, diet designed to meet 100% of MP requirements; MP80, diet designed to meet 80% of MP requirements; MP60, diet designed to meet 60% of MP requitrements. Diets formulated based on a 70-kg ewe carrying twins.

Histological traits

There was not a MP level × fetal number interaction ($P \geq 0.15$) or main effects of MP level ($P \geq 0.21$) or fetal number ($P \geq 0.79$) for fetal BNC number or percentage occupancy of the COT. There was however, an interaction ($P = 0.04$) for BNC size. In MP60 ewes carrying twins, there was a greater BNC size compared to all other treatment groups, except the MP100 ewes carrying singletons that did not differ ($P = 0.10$; Figure 1). While there was not an interaction of the proportion of the placentome occupied by COT ($P = 0.41$) or a main effect of MP level ($P = 0.43$), there was an increase ($P < 0.01$) in the percentage of COT in twin vs. singleton pregnancies (58.63 vs. 51.94 ± 1.47%). Binucleate cells release steroid hormones such as progesterone (Wooding, 1992). Previously, our lab observed that ewes carrying twins had greater progesterone and estradiol-17β in circulation compared to singletons (Swanson et al., 2017). This could simply be from the overall greater placentome mass that the twins had. We did not measure local concentrations of steroids in fetal circulation, but our BNC data would lead us to hypothesize the fetuses of MP60 ewes carrying twins would have greater concentrations. We have also reported that MP60 carrying singletons (we

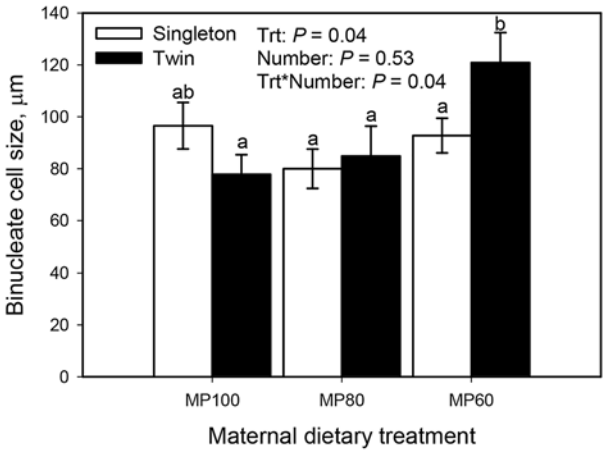

Figure 1. Impacts of maternal metabolizable protein (MP) level and fetal number on binucleate cell size in the placentome at d 130 of gestation in sheep.

did not have twins in that study; Lekatz et al., 2015) had greater uterine blood flow near term. Perhaps if we examined uterine blood flow to the gravid horn of MP60 ewes with twins, we would have seen further compensations for these protein restricted dams.

IMPLICATIONS

While we did not alter BNC numbers, we did observe that BNC size was increased in our MP60 ewes carrying twins, indicating that the placenta may try to compensate when there are multiple stressors during pregnancy. These multiparous ewes may have adapted to unfavorable environments by providing more nourishment by factors produced by BNCs. Studies are underway to investigate the factors that are made in BNCs and how those change when stressful situations are encountered. If we know what factors may be limiting, perhaps there would be a way to provide those factors to at-risk pregnancies in dams.

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The effects of feeding reduced-lignin alfalfa on growing beef cattle performance[1]

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ABSTRACT: Digestibility of forage cell walls is limited by interactions between structural and non-structural polysaccharides such as lignin, hydroxycinnamic acids, protein, ions, and water. Gene suppression techniques down-regulating caffeoyl CoA 3-*O*-methyltransferase (CCOMT), a specific lignin biosynthesis pathway enzyme, have permitted the development of a more digestible variety of alfalfa known as HarvXtra (HX-4114). Dairy cattle and sheep exhibited an increase in production when consuming the reduced-lignin alfalfa compared to conventional alfalfa in preliminary studies. Therefore, a study was designed to evaluate the effects of feeding a reduced-lignin alfalfa (HX-4114) versus a conventional alfalfa (variety WL336HQRR) on growing beef cattle performance. Twenty-four heifers (initial BW = 270 ± 21 kg) were allocated, three heifers per pen (n = 3), to receive either reduced-lignin (R; n = 12), or conventional variety alfalfa hay (C; n = 12) for an 84-day feeding trial. The trial consisted of four 28-d periods. Alfalfa was planted in June 2016 under center-pivot irrigation, and the second harvest was used for the feeding trial. Heifers were fed using GrowSafe feeders to track individual daily feed intake. Heifers were fed daily at 3.75% BW. Hay samples were collected weekly to evaluate forage quality. There were no differences ($P \geq 0.05$) in forage quality between treatments, except for DM ($P = 0.01$). There were no treatment or treatment by day interactions ($P \geq 0.05$) for BW, ADG, or DMI. The average initial and final BW for the reduced-lignin treatment was 269.95 ± 3.79 kg and 367.24 ± 3.79 kg, respectively. The average initial and final BW for the conventional treatment was 270.19 ± 3.79 kg and 362 ± 3.79 kg, respectively. Although there were no differences between measured treatment parameters, heifers receiving the reduced-lignin alfalfa weighed 4.25 kg more at the end of the trial than heifers receiving the conventional alfalfa treatment. This could be economically advantageous for beef cattle producers. Additional research is needed to further explore the quality and value of feeding reduced-lignin alfalfa on growing beef cattle performance, and its potential impact on the U.S beef industry.

Key words: alfalfa, beef, cattle, HarvXtra, performance, reduced lignin

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INTRODUCTION

Forages are the basis of many livestock rations and are the main energy source for the grazing ruminant animal. Ruminants depend on dietary carbohydrates, found in the cell wall of forage, for over half the energy needed for maintenance, growth, and production (Nafikov and Beitz, 2007). Unfortunately, the fraction of cell wall in forage that is readily digested and utilized by the animal is less than 50% (Hatfield et al., 1999). Land plants have evolved over time by developing a rigid cell wall that provides mechanical strength and hydrophobicity, as well as resistance to degradation by herbivores, micro-organisms, and enzymes (Wilson, 1994; Theodorou et al., 1996; Hatfield et al., 1999). These cell walls comprise 20-80% of forage dry weight and are composed of structural and non-structural polysaccharides, lignin, hydroxycinnamic acids, protein, ions, and water (Wilson, 1994; Hatfield et al., 1999) Interactions between these components influence forage quality and consequently, animal performance (Hatfield et al., 1999).

Ruminants produce 70% of the total animal protein and 10% of the total fiber consumed by humans

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(Nafikov and Beitz, 2007). Improving production efficiency of domestic ruminants for human consumption requires improvements in both animal genetics and forage cell walls. Many years of forage research have been dedicated to finding the molecular mechanism that controls cell wall degradation in order for a greater percentage of potential energy to be made available to the animal.

Innovative technology using gene suppression techniques to down-regulate caffeoyl CoA 3-*O*-methyltransferase (CCOMT), a specific lignin biosynthesis pathway enzyme, has permitted the development of a more digestible variety of alfalfa known as HarvXtra (HX-4114; McCaslin et al., 2014). Compared to related null lines of alfalfa, HarvXtra has a 15-20% reduction in lignin content and a 10-15% increase in neutral detergent fiber digestibility (NDFD) and relative forage quality (RFQ; McCaslin et al., 2014).

Published data for animal feeding trials using HarvXtra alfalfa are lacking but progress reports are available. Mertens (2009) found that down-regulating lignin in alfalfa significantly improved fiber digestion ($P < 0.01$) with daily gains for lambs receiving low lignin treatments being 10% higher than lambs consuming conventional alfalfa.

Comparing the results from brown midrib (BMR) forage feeding trials to reduced-lignin alfalfa feeding trials is applicable because the BMR mutant produces plants with lower lignin content and higher IVDMD. In vitro and in situ NDF digestion trials using BMR mutant forages found BMR forages to have lower lignin content and higher NDF digestibility than their nulls (Rook et al., 1977; Keith et al., 1979; Cherney et al., 1991). Studies using BMR varieties report increased DMI, improved milk production, and better weight gain (Rook et al., 1977; Aydin et al., 1999).

Our objectives were to determine the differences in growing heifer performance when diets consisted of either a reduced-lignin or conventional alfalfa variety. Our hypothesis was that animals consuming reduced-lignin alfalfa would have higher ADG and higher final BW compared to animals consuming conventional varieties of alfalfa.

MATERIALS AND METHODS

All protocols for this study were reviewed and approved by the Montana State University Agricultural Animal Care and Use Committee (#2016-AA15).

Forage Establishment and Harvest

A variety of reduced-lignin alfalfa (HX-4114) and a variety of conventional alfalfa (WL336HQRR) were planted south of Townsend, MT, on May 19, 2016. Soil samples were collected prior to planting and laboratory results for nutrient analysis were used to determine and apply appropriate fertilization. Approximately 3.6 kg pure live seed/ ha was drilled using a Great Plains 3S-3000HD double disc drill at a 1.27 cm depth, spaced 15 cm apart, into a firm, weed-free seedbed. Both varieties were planted in the same field, each on 8 ha. The field was irrigated with a center-pivot already in place at the research site. First and second harvests were taken on July 29, 2016, and October 10, 2016, respectively, at approximately 10% bloom. Production for the second harvest, which was used to feed the animals in this study, totaled 2,951 kg/ha and 2,908 kg/ha for the conventional and reduced-lignin varieties, respectively.

Feeding Trial

Twenty-four crossbred Angus heifers (initial BW = 270 ± 21 kg) were utilized in a completely randomized design in an 84-day trial beginning on November 14, 2016, and ending on February 7, 2017. Heifers were stratified by BW to one of two alfalfa varieties (n = 3 heifers/pen). Each pen was equipped with a GrowSafe System (GrowSafe Systems Ltd.) to determine individual heifer intake. Heifers were fed at 3.75% BW on a DM basis and had ad libitum access to water and Easylix 12-12-12 mineral pressed blocks.

Heifers were acclimated to their respective treatments 10 d prior to the start of the trial. No data were recorded during this time. Heifers were weighed immediately prior to the start of the trial on d -1 and 0, and every 28 d thereafter. Weights were taken for two consecutive d, and individual BW were averaged to obtain individual BW by period. Sampling occurred on days -1, 0, 27, 28, 55, 56, 83, and 84.

Forage Analysis

Forage samples for each treatment were taken weekly and two weeks were combined for each treatment, totaling seven composited samples per treatment. Samples were ground in a Wiley mill to pass a 2-mm screen and sent to a commercial lab (Cumberland Valley Analytical Services, Hagerstown, MD) to be analyzed for DM, NDF, ADF, CP (FP528 analyzer, LECO Corporation, St. Joseph, MI), relative feed value (RFV), RFQ, TDN, and NDFD48.

Statistical Analysis

Data were analyzed as a completely random design using the PROC MIXED procedure of SAS (version

9.4; SAS Inst. Inc., Cary, NC), with heifer as the experimental unit. Treatment and period were set as fixed effects, with heifer as a random effect. Means were separated using the LSMEANS procedure of SAS and *P*-values of ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Forage quality parameters are reported in Table 1. There was a difference between treatments in DM ($P = 0.01$). There were no significant differences between treatments in CP ($P = 0.69$), ADF ($P = 0.11$), NDF ($P = 0.60$), lignin ($P = 0.25$), NEg ($P = 0.31$), RFV ($P = 0.47$), RFQ ($P = 0.53$), TDN ($P = 0.18$), or NDFD48 ($P = 0.39$).

Main effects of alfalfa treatment on BW, ADG, and DMI during the 84-day study period are presented in Table 2. No treatment by day interactions ($P \geq 0.05$) were observed. Treatment did not affect ($P = 0.35$) BW at d 0, 28, 56, or 84. Treatment did not affect ADG ($P = 0.32$), or DMI ($P = 0.82$) for d 0-28, 29-54, or 55-84.

TABLE 1. Forage quality parameters for reduced-lignin and conventional alfalfa

Nutrient composition[1]	Treatment	
	Reduced lignin	Conventional
DM	92.23 ± 0.13[a]	92.87 ± 0.13[b]
CP	20.51 ± 0.32	20.70 ± 0.32
ADF	24.17 ± 0.54	25.61 ± 0.54
NDF	30.61 ± 0.72	31.17 ± 0.72
Lignin	5.69 ± 0.19	6.02 ± 0.19
NEG	0.40 ± 0.005	0.39 ± 0.005
RFV	213.71 ± 5.82	207.43 ± 5.82
RFQ	218 ± 6.31	212 ± 6.31
TDN	64.67 ± 0.33	63.96 ± 0.33
NDFD48	47.44 ± 0.72	46.49 ± 0.72

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

[1]NEG- net energy-gain; RFV- relative feed value; RFQ- relative feed quality; NDFD48- neutral detergent fiber digestibility at 48 hours.

Heifers in this study were fed at 3.75% BW, permitting each heifer full access to feed. This protocol was implemented to ensure adequate intake and to warrant a practical feeding application. Further research is needed to explore the effects of feeding reduced-lignin alfalfa in a restricted feed setting, along with similar research using cattle of various ages.

Although there were no significant differences between the measured treatment parameters, heifers receiving the reduced-lignin alfalfa weighed 4.25 kg more at the end of the trial than heifers receiving the conventional alfalfa treatment. This could be economically advantageous for beef cattle producers. For example, if we use 2017 feeder heifer prices (USDA-AMS, 2017; $115-128 per cwt) and an average heifer weight of 363 kg, the feeder heifers will be worth $2.64 per kg. If these feeder heifers gain an average of 4.25 kg more when consuming reduced-lignin alfalfa, this will equate to $11.22 more per heifer. A producer who sells 100 heifers will earn $1,122 more than a producer who feeds conventional alfalfa.

One noticeable observation during this study was the lower ADG during d 57-84 compared to the ADG for d 0-28 and d 29-56. One possible explanation for this outcome may be extreme temperatures and cold stress. The heifers in this study were exposed to temperatures outside their thermoneutral zone for extended periods of time during d 57-84. The thermoneutral zone for healthy beef cattle is between 0º and 25º C. Cattle exposed to temperatures within this range do not have to expend energy to maintain normal body temperature. The low and high temperatures for the month of January were -33º and 7.22º C, respectively, with an average of -12º C (Underground, 2016). Houseal and Olson (1995) concluded that animals exposed to lower critical temperatures (-13º to -47º C depending on size, sex, age plane of nutrition, and previous acclimatization) for prolonged periods of time have the potential to become cold stressed and must increase metabolic heat production to maintain homeostasis. Birkelo et al. (1991) found that cold in-

TABLE 2. LS means and standard error for BW, ADG, and DMI across dates and treatments

	Trait[1]					
	BW		ADG		DMI	
Day	R	C	R	C	R	C
0	269.95 ± 3.79	270.19 ± 3.79	---	---	---	---
28	302 ± 3.79	299.75 ± 3.79	1.14 ± 0.06	1.06 ± 0.06	8.64 ± 0.53	8.66 ± 0.53
56	338.23 ± 3.79	334.39 ± 3.79	1.29 ± 0.06	1.24 ± 0.06	10.47 ± 0.53	10.04 ± 0.53
84	367.24 ± 3.79	362.99 ± 3.79	1.04 ± 0.06	1.02 ± 0.06	11.26 ± 0.53	11.19 ± 0.53
Avg	319.35 ± 3.51	316.82 ± 3.51	1.16 ± 0.04	1.10 ± 0.04	10.12 ± 0.48	9.96 ± 0.48

[1]R-reduced lignin; C- conventional.

creased requirements for weight maintenance or gain and that acute cold stress resulted in lower performance of feedlot animals during the colder parts of the year. It is quite possible that the animals in this study were cold stressed during the month of January and the beginning part of February due to prolonged cold temperatures, resulting in lower ADG.

IMPLICATIONS

Differences between treatments in this study were not significant; however, the BW data are indicative of improved animal performance for animals being fed reduced-lignin alfalfa for prolonged periods. Additional research is needed to further explore the quality and value of feeding reduced-lignin alfalfa on beef cattle performance and its potential impact on the U.S beef industry.

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Glycerin supplementation via drinking water alters nitrogen balance and immune response of beef steers during an endotoxin challenge[1]

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ABSTRACT: This experiment evaluated innate immunity, diet digestibility and N balance of steers in response to a lipopolysaccharide (LPS) challenge and glycerin supplementation in drinking water. Steers (n = 24; 203 ± 3.8 kg BW) were blocked by BW (2 blocks) and randomly assigned to 4 treatment combinations in a randomized complete block design with a 7-d adaptation period and 5-d collection period. Treatments were a 2 × 2 factorial arrangement of crude glycerin added to the drinking water at 0 (-GLY) or 25 g/L (+GLY), and LPS dissolved in 2 mL of sterile saline injected subcutaneously on d 8 to supply either 0 (-LPS) or 3 µg (+LPS) of *Escherichia coli* O55:B5 per kg of BW. Immune response was measured by changes in rectal temperatures and serum concentrations of tumor necrosis factor-α (TNF-α), IL-6, and cortisol collected at 0, 2, 4, 8, and 12 h after LPS injection. Feed intake, fecal samples, and urine collections determined diet digestibility and N balance. All variables were analyzed using the MIXED procedure of SAS. Interactions for LPS × GLY × h ($P \leq 0.02$) occurred for rectal temperature, TNF-α, and IL-6. Steers receiving both +LPS and +GLY had greater rectal temperatures (2 and 4 h), TNF-α (2 h), and IL-6 (2, 4, and 8 h) compared with the other treatment combinations. An LPS × h interaction ($P < 0.01$) was observed for serum cortisol. Steers receiving +LPS had greater cortisol concentrations at 2, 4, and 8 h than -LPS steers. Retained N was lower ($P < 0.01$) for +LPS than –LPS steers. Steers receiving +GLY retained less ($P = 0.05$) grams of N than -GLY steers. There was a tendency for lower ($P \leq 0.10$) DMI and total tract NDF digestibility in +GLY compared to -GLY steers. Water intake, ADF digestibility, and N digestibility were not different ($P \geq 0.22$) among treatments. These results indicate that LPS causes physiological stress and that supplementing glycerin via drinking water during an LPS challenge results in a greater innate immune response. However, adding glycerin to drinking water at 25 g/L negatively impacted diet digestibility in steers. Stressed steers may benefit from supplemental glycerin via drinking water when exposed to disease because of a more robust immune system.

Key words: cattle, cytokines, digestibility, glycerol, lipopolysaccharide
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INTRODUCTION

Consumer pressure to eliminate metaphylactic antibiotic use may increase the need for alternative strategies to address illness in receiving cattle. With or without antibiotics, Duff and Galyean (2007) suggested that a stronger immune system, and subsequently better performance, may be possible through increased caloric intake. Crude glycerin (**GLY**), a liquid byproduct from the biodiesel industry, is considered a high-energy product safe for animal consumption (if less than 150 ppm methanol). Glycerin has been used to replace grain in finishing cattle diets (Parsons et al., 2009; Bartoň et al., 2013; Buttrey et al, 2015).

Because of low DMI by morbid calves (Sowell et al., 1999), nutrients supplemented via feed may not be consumed in large enough quantities to sat-

[1]Authors thank High Plains Bioenergy (Guymon, OK) for the donation of crude glycerin used in this study and Dr. Michael Hubbert for guidance.
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isfy the requirements for stressed animals (Hales et al., 2013). Therefore, nutrients may need to be supplied by an alternative route before the potential benefits of supplemental nutrients can be realized. Garza and Owens (1989) suggested drinking water as a carrier for essential nutrients, and Buhman (2000) reported that morbid calves drink more frequently in the first few days of arrival than their healthy counterparts.

We hypothesized that glycerin supplementation via drinking water will be beneficial for the innate immune system, but may also alter total tract diet digestibility and N balance of steers. The objectives of this study were to evaluate the effects of glycerin supplementation via drinking water on the innate immune response and diet digestibility in steers exposed to lipopolysaccharide (**LPS**).

MATERIALS AND METHODS

Animals and Facilities

The New Mexico State University Institutional Animal Care and Use Committee approved (# 2016-029) all procedures according to the NRC (2011). Twenty-four ruminally cannulated beef steers (203 ± 3.8 kg BW) were housed in individual tie stalls with rubber mats in a metabolism facility with continuous lighting and evaporative cooling (22.6 ± 2.03°C). Steers were fed a diet (Table 1) twice daily (0700 and 1900 h) in equal portions totaling 1.7% of BW (DM basis).

Experimental Design and Treatments

The experiment was a randomized complete block design with a 7-d adaptation period and 5-d collection period. Steers were blocked by BW into 2 blocks (initial BW were 214 ± 4.4 and 193 ± 4.5 kg for blocks 1 and 2, respectively) with 12 steers per block. Treatments were a 2 × 2 factorial arrangement of crude glycerin added to drinking water at 0 (**-GLY**) or 25 g/L (**+GLY**), and LPS dissolved in 2 mL of sterile saline injected subcutaneously in the neck to supply either 0 (**-LPS**) or 3 µg (**+LPS**) of *Escherichia coli* O55:B5 (Sigma Chem. Co., St. Louis, MO) per kg of BW. The LPS treatments were injected at 0900 h on d 8 of the experiment. Calves had ad libitum access to individual water treatments throughout the 12-d experiment. The concentration of crude glycerin (Table 2) added to drinking water was determined during a previous experiment using sheep as a model (Pillmore et al., 2017).

TABLE 1. Ingredient and nutrient composition of diets

Item	% of DM
Ingredient	
Corn grain, cracked	35.9
Alfalfa hay, chopped	30.0
Dried distiller's grains	15.0
Beet pulp with molasses	10.0
Molasses	8.0
Supplement[1]	1.12
Nutrient[2]	
NDF	26.1
ADF	17.7
Crude protein	15.1
K	1.90
Ca	0.75
P	0.33
Mg	0.22

[1]Supplied (DM basis): 0.50% urea, 0.30% salt, 0.25% limestone, 3.60 ppm Cu, 8.75 ppm Fe, 0.07 ppm Se, 18.00 ppm Zn, 3,000 IU/kg vitamin A, 600 IU/kg vitamin D, 150 IU/kg vitamin E, 34 mg/kg monensin.

[2]Analyzed by SDK Laboratories (Hutchinson, KS).

TABLE 2. Composition of crude glycerin

Item[1]	% as fed
Glycerol	84.9
Water	7.9
Ash	6.3
Many organics non-glycerol (MONG)	0.87
Free fatty acids	0.10
Methanol	<0.01

[1]Analyzed by SDK Laboratories.

Collections

During the 5-d collection period, water intake was measured by weight. Blood samples were collected at 0, 2, 4, 8, and 12 h relative to LPS administration via an indwelling jugular catheter (J-457A; Jorgensen Laboratories, Loveland, CO), inserted on d 6 of the experiment. At each time point, blood samples were collected into disposable syringes (Monoject; Medtronic, Minneapolis, MN) and transferred to vacuum tubes (Corvac serum separator; Medtronic). Samples were allowed to coagulate at room temperature for 30 min then centrifuged (TJ-6; Beckman Coulter Life Sciences, Indianapolis, IN) at 1,500 × *g* for 20 min at 10°C. Serum was transferred into 7-mL vials and stored at -20°C until analysis. Rectal temperatures were measured before LPS injection (0 h) and 2, 4, 8, 12, and 24 h after LPS injection using a digital thermometer (Model 144-691-000; ReliOn, Bentonville, AR).

To calculate diet digestibility and fecal output, chromic oxide (8 g) was dosed via the rumen cannula twice daily during the 12-d experiment as described

by Islas and Soto-Navarro (2011). Fecal samples were obtained from the rectum every 4 h; times of fecal collection were advanced by 1 h for each day of collection such that every h in a 24-h period was represented over the 5-d collection period. Fecal samples were later composited by steer, dried in a forced-air oven (Model POM-326F, Blue M Electric Company, Blue Island, IL) at 55°C, allowed to air equilibrate, ground through a 2-mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ) and stored until analysis. Diet samples were collected on d 1 of each period. Feed refusals were collected daily during the 5-d collection period at 0700 h (before feeding), weighed, and frozen for later analysis. Daily urine was collected into 20-L vessels (No. C14907, Nasco, Modesto, CA) using vacuum pumps (DOA-P704-AA, Gast, Benton Harbor, MI) connected to rubber urine pouches (Itran Rubber Co., South Plainfield, NJ) harnessed to steers. To minimize NH_3 volatization, each vessel contained 300 mL of 3 *M* HCl. Harnesses were attached 12 h after LPS administration so as not to interfere with serum analysis of cortisol, an indicator of stress. Total urinary output was weighed daily during the last 4 d of the collection period at 1900 h, and representative samples of 1% were stored at -20ºC and composited by steer for analysis.

Sample Analysis

Serum samples were analyzed for cortisol concentrations using RIA (intra-assay CV = 4.3%; Anti-Cortisol Antibody Coated Tubes; MP Biomedicals LLC, Solon, OH) by the New Mexico State University Endocrinology Laboratory. Serum concentrations of tumor necrosis factor-α (**TNF-α**) and IL-6 were analyzed using ELISA as described by Carroll et al. (2009).

Feces, diet, and feed refusals were analyzed for DM, NDF, ADF, and N concentrations, and urine was analyzed for Kjeldahl N by a commercial laboratory (SDK Laboratories). Chromium concentration in the feces was determined by first wet ashing the samples to liberate the Cr from the Cr_2O_3, then using spectrometry to analyze for Cr by a commercial laboratory (SDK Laboratories). Daily fecal DM output was determined by dividing 10.95 g Cr (daily dose supplied by Cr_2O_3, assuming 68% Cr) by the concentration of Cr in the feces.

Statistical Analysis

All data were analyzed using MIXED procedure of SAS (9.0; SAS Inst. Inc., Cary, NC) as a randomized complete block design. The experiment was blocked by BW, and steer was the experimental unit. When dependent variables did not have repeated observations, the fixed effects of the model were LPS, GLY, and LPS × GLY interaction with block and steer as random effects. Variables with repeated measures were modeled to include h, block, steer, LPS, and GLY as sources of variation with steer and block within treatment combination (LPS × GLY) as random effects. All possible interactions of LPS, GLY, and h were included in the model. Differences were considered significant at $P \leq 0.05$, and $P \leq 0.10$ were considered tendencies.

RESULTS

Interactions of LPS and GLY

Interactions between LPS, GLY, and h ($P \leq 0.02$) were observed for serum TNF-α and IL-6 (Fig. 1), and rectal temperatures (Fig. 2). Serum concentrations of TNF-α for +LPS steers receiving +GLY increased from 0 to 2 h, were greater than all other treatment combinations at 2 h, then decreased from 2 to 4 h and were not different among treatments at 4, 8, and 12 h after LPS injection. Serum concentrations of IL-6 increased from 0 to 2 h for +LPS steers, and were greater than -LPS steers at 2 and 4 h, with greater concentrations for +LPS steers receiving +GLY than –GLY at 2, 4, and 8 h. Rectal temperatures of +LPS steers increased from 0 to 2 h, and were greater than -LPS steers at 2 and 4 h, with temperatures of +LPS steers receiving +GLY higher than –GLY at 2 and 4 h. No LPS × GLY interactions ($P \geq 0.14$) occurred for water intake, DMI, fecal excretion, nutrient digestibility, or N balance, except for urinary N excretion that exhibited an LPS × GLY interaction ($P = 0.05$; Table 3). Supplementation of glycerin did not alter the already high urinary N excretion observed for +LPS steers, but when steers did not receive LPS, +GLY steers excreted greater N than -GLY steers.

Effects of LPS

An LPS × h interaction was observed for serum cortisol ($P < 0.01$; Fig. 3); cortisol concentrations increased from 0 to 2 h and were elevated for +LPS compared to -LPS steers at 2, 4, and 8 h after LPS injection. Intake, fecal excretion, and digestibility of DM, NDF, ADF, and N, and water intake, were not affected by LPS ($P \geq 0.11$), but +LPS steers had lower ($P < 0.01$) N retention than -LPS steers (Table 3).

Effects of GLY

Glycerin supplementation tended to be lower ($P \leq 0.10$) for DM and N intake, total tract DM and NDF digestibility, and N retention (as percent of N intake) compared to -GLY steers (Table 3). Steers sup-

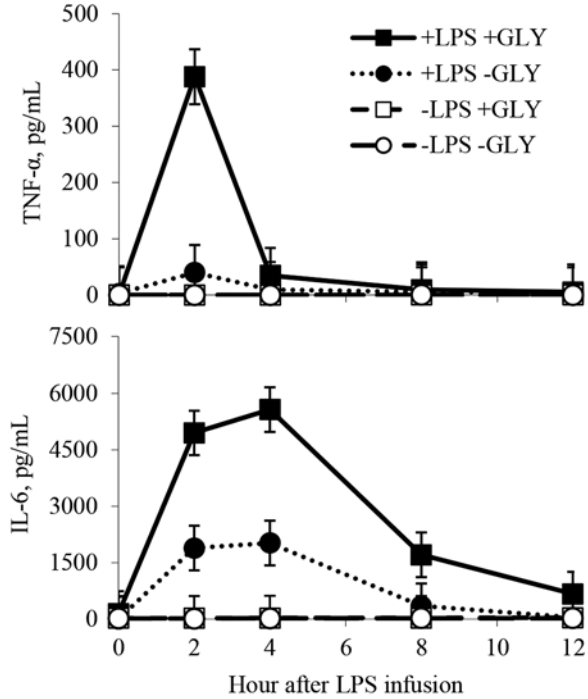

Figure 1. Serum tumor necrosis factor-α (TNF-α) and IL-6 concentrations of steers in response to lipopolysaccharide (LPS) in a subcutaneous injection to supply 0 or 3 µg (-LPS vs. +LPS) per kg of BW, and crude glycerin added to drinking water at 0 or 25 g/L (-GLY vs. +GLY). Effects were: LPS × GLY × h ($P \leq 0.02$), LPS × h ($P < 0.01$), and GLY × h ($P \leq 0.02$).

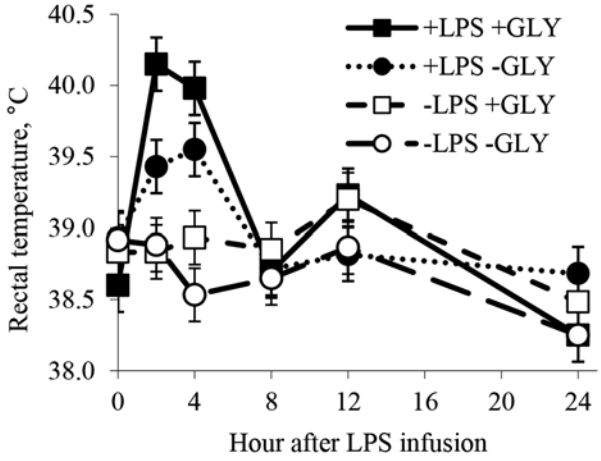

Figure 2. Rectal temperatures of steers in response to lipopolysaccharide (LPS) in a subcutaneous injection to supply 0 or 3 µg (-LPS vs. +LPS) per kg of BW, and crude glycerin added to drinking water at 0 or 25 g/L (-GLY vs. +GLY). Effects were: LPS × GLY × h ($P = 0.02$), LPS × h ($P < 0.01$), and GLY × h ($P < 0.01$).

plemented with glycerin had lower ($P = 0.05$) daily grams of retained N than -GLY steers. Administration of GLY did not affect ($P \geq 0.22$) ADF digestibility, N digestibility or water intake.

DISCUSSION

Increased serum cortisol concentrations within the first 2 h of LPS injection is consistent with Carroll et al. (2009) and Waggoner et al. (2009), and is indicative of physiological stress. Stimulation of the innate immune system with LPS causes an increase in pro-inflammatory cytokines which leads to a greater febrile response (Carroll and Burdick Sanchez, 2014). Gluconeogenic effects of glycerin (Lin, 1977) could further increase pro-inflammatory cytokine synthesis (Carroll and Burdick Sanchez, 2014), which may account for greater TNF-α, IL-6, and rectal temperatures when endotoxin-challenged steers received +GLY than –GLY. These results may, in part, explain the greater rectal temperatures often observed in feedlot receiving calves consuming high-energy, high-concentrate diets compared to low-energy, high-roughage diets (Duff and Galyean, 2007). Although elevated rectal temperatures are generally a diagnostic tool for morbidity, Duff and Galyean (2007) also speculated that greater body temperatures could be indicative of increases in pro-inflammatory cytokines. Therefore, it is plausible that the additional energy from glycerin supplementation in drinking water could better equip highly-stressed feedlot receiving calves to fight infection.

Crude glycerin supplementation via drinking water may have increased the innate immune response of endotoxin-challenged steers, but it also tended to decrease DMI, even when steers were limit-fed at 1.7% of BW (DM basis). Parsons et al. (2009) also observed a linear decrease in DMI when up to 16% crude glycerin (DM basis) replaced steam-flaked corn in finishing heifer diets. These decreases in DMI might suggest an effect of glycerin on rumen microbial fermentation. In a companion paper, Lopez et al. (2017) observed decreased ruminal acetate and increased ruminal propionate production in steers supplemented with crude glycerin via drinking water. According to Allen (2000), greater propionate production could decrease DMI. Lower NDF digestibility associated with glycerin-supplemented steers is consistent with lower ruminal acetate-to-propionate ratios reported by Lopez et al. (2017).

Previous studies indicate that immune-challenged calves have lower N retention than their counterparts, likely due to increased protein catabolism to mount an immune response (Waggoner et al., 2009). The results of this study indicate that glycerin supplementation via drinking water did not alleviate protein catabolism associated with an endotoxin challenge.

IMPLICATIONS

A greater innate immune response of endotoxin-challenged steers when supplemented with crude glycerin

Table 3. Intake, excretion, and digestibility of nutrients for beef steers in response to crude glycerin (GLY) added to drinking water and bacterial lipopolysaccharide (LPS) subcutaneous injection

	Treatments[1]							
	-LPS		+LPS			*P*-value		
Item	-GLY	+GLY	-GLY	+GLY	SEM[2]	LPS × GLY	LPS	GLY
Steers	5	6	6	6				
Intake, g/d								
DM	3396	3378	3397	3055	113	0.14	0.14	0.10
NDF	885	881	884	800	29.0	0.15	0.14	0.12
ADF	601	599	601	541	20.6	0.14	0.15	0.12
N	82.2	81.6	82.1	73.5	2.78	0.14	0.13	0.09
Water intake, L/d	11.9	16.8	12.2	13.2	2.66	0.48	0.54	0.28
Fecal, g/d								
DM	1232	1475	1374	1309	109	0.14	0.90	0.39
NDF	655	719	648	640	46.4	0.41	0.33	0.52
ADF	467	473	428	410	32.7	0.69	0.11	0.85
N	35.2	38.8	38.7	37.7	3.76	0.52	0.73	0.71
Urinary N, g/d	20.3[a]	29.3[ab]	33.8[ab]	28.5[b]	3.73	0.05	0.08	0.60
Digestibility, %								
DM	63.8	56.3	59.5	57.7	2.73	0.24	0.56	0.07
NDF	26.1	18.5	26.7	20.6	4.10	0.84	0.71	0.09
ADF	22.5	21.0	28.7	24.9	4.32	0.77	0.22	0.51
N	57.1	52.3	52.8	49.7	3.88	0.80	0.35	0.28
Retained N								
g/d	26.6[a]	13.4[ab]	9.5[ab]	7.3[b]	4.10	0.16	<0.01	0.05
% of N intake	32.3[a]	16.3[b]	11.6[b]	9.1[b]	5.13	0.17	<0.01	0.07

[a,b]Means in rows with different superscripts differ ($P \leq 0.05$).

[1]Treatments were a 2 × 2 factorial arrangement of crude glycerin added to drinking water at 0 (-GLY) or 25 g/L (+GLY), and LPS dissolved in 2 mL of a sterile subcutaneous saline injection to supply either 0 (-LPS) or 3 µg (+LPS) of Escherichia coli O55:B5 (Sigma Chem. Co., St. Louis, MO) per kg of BW.

[2]For 5 steers per treatment.

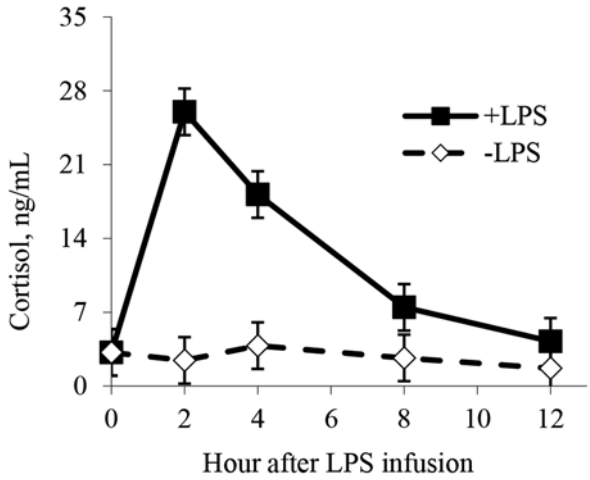

Figure 3. Serum cortisol concentrations of steers in response to lipopolysaccharide (LPS) in a subcutaneous injection to supply 0 or 3 µg (-LPS vs. +LPS) per kg of BW. Effects were LPS × h ($P < 0.01$).

via drinking water implies that the supply of additional energy from glycerin may better prepare immune-compromised calves to fight infections. However, glycerin supplementation via drinking water may negatively affect ruminal fiber digestion, feed intake, and N retention of growing cattle, regardless of their immune status.

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Effect of supplementation during the breeding season on a May-calving herd in the Nebraska Sandhills

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ABSTRACT: A 4-yr study was conducted at Gudmundsen Sandhills Laboratory near Whitman, NE, to determine the effects of supplementation during the breeding season on yearling and primiparous crossbred (5/8 Red Angus, 3/8 Continental), May-calving heifers. Heifers were utilized in their first and second breeding season. Throughout the breeding season, heifers and primiparous heifers grazing upland range were offered either no supplement (NS, n = 128 or 67; heifers or primiparous heifers, respectively) or a 32% CP (DM) supplement delivered 3 time/wk on a pasture basis. Heifers (304 ± 2 kg) received 0.45 kg/d supplement (SUP, n = 129), and primiparous heifers (387 ± 3 kg) received 0.91 kg/d supplement (SUP, n = 68). For the remainder of the year, females were managed as a single herd within their respective age group. Heifers and primiparous heifers were synchronized using a single PGF2a injection 5 d after bull placement (1:20 bull to heifer ratio) for a 45 d breeding season. Pregnancy was diagnosed via transrectal ultrasonography a minimum of 45 d following bull removal. Calves were weaned at pregnancy diagnosis. Heifer and primiparous heifer BW and BCS was recorded prior to breeding, at pregnancy diagnosis, and prior to calving. Calf BW was recorded at birth, prior to start of the breeding season, and at weaning. Supplemented heifer calves tended ($P = 0.10$) and primiparous heifers had greater ($P < 0.01$) BW at pregnancy diagnosis. However, pregnancy rate in heifers and primiparous heifers were similar ($P \geq 0.41$) between treatment groups. Supplementation during the breeding season did not affect dystocia rates ($P \geq 0.79$) or calf birth BW ($P \geq 0.05$) in either age group. First calf weaning weight was greater for SUP dams ($P < 0.01$). However, second calf weaning weight was similar ($P = 0.47$) and not influenced by previous year's treatment. In summary, supplementation during the breeding season did not affect pregnancy rates in young beef females, despite changes in BW in heifers and primiparous heifers. An increase in weaning BW of calves nursing SUP dams was observed. Further research is needed to determine if a greater rate of supplementation is needed to elicit a response in pregnancy rates.

Key words: beef heifer, May calving, reproduction, supplementation
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INTRODUCTION

In the northern Great Plains, early summer calving herds better match forage quality to peak nutrient requirements. Early lactation occurs when CP and DE are greatest, thus providing abundant energy and requiring fewer harvested feed inputs (Stockton et al., 2007). Research by Griffin et al. (2012) demonstrated similar pregnancy rates among cows in 3 different calving systems (May, June, and August); however, younger females exhibited a decreased pregnancy rate in a May vs March calving system (70 vs. 89%, respectively; Springman et al., 2017). Forage seasonality (warm vs. cool season), precipitation levels, and ambient temperature affect the quality and quantity of forage available during the breeding season. Forage quality tends to decline into late summer and remains low through winter (Randel, 1990). Later breeding seasons occur in periods of greater ambient temperature; however, temperature does not appear to be a limiting factor in older beef females in the Nebraska Sandhills as Griffin et al. (2012) found similar pregnancy rates in 3 different calving groups. It is more likely the capacity of younger females to physically consume enough lower quality forage that impacts pregnancy rates in younger beef cows in a later breeding season (Funston

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et al., 2016). Inadequate protein or energy after calving and during the breeding season decreases pregnancy rates and extends the length of the postpartum interval (Stockton et al., 2007). The objective of this study was to determine effects of supplementing May-calving heifers and primiparous beef heifers during the breeding season on ADG and reproductive response.

MATERIALS AND METHODS

The University of Nebraska Animal Care and Use Committee approved the procedures and facilities used in this experiment.

Heifers

A 4-yr study conducted at Gudmundsen Sandhills Laboratory near Whitman, NE, utilized May-born, crossbred (5/8 Red Angus, 3/8 Continental) replacement heifers (n = 257). Heifers were randomly assigned to receive either no supplement (**NS**) or offered 0.45 kg/d of supplement (**SUP**; 32% CP, DM) beginning 2 wk prior to and throughout a 45 d breeding season. Heifers grazed upland range in addition to supplementation. Supplement was delivered 3 times/wk on a pasture (35.6 ha) basis.

Heifers were blocked by previous winter treatment (Springman et al., 2017) and assigned to breeding treatment.

Preceding the breeding season, BW was recorded and blood samples collected at d -10 and d 0 of the breeding season. A heifer with plasma progesterone concentration of greater than 1 ng/ml at either collection was considered pubertal.

Approximately July 22, bulls were placed with heifers (1:20 bull to heifer ratio) for 45 d. Heifers were synchronized using a single $PGF_{2\alpha}$ (Lutalyse, Zoetis, Florham Park, NJ) injection 5 d after bulls were introduced. After the supplementation period, heifers were managed as a single herd and grazed upland Sandhills range. Pregnancy was diagnosed via transrectal ultrasonography (Aloka, Hitachi Aloka Medical America Inc., Wallingford, CT) and BW and BCS measured in October, a minimum of 45 d following bull removal.

Prepartum BW and BCS was measured 14 d prior to an expected calving due date of May 2. The first day 2 or more heifers calved was considered the start of the calving season, and was used to calculate percent calved in the first 21 d. A calving ease (**CE**) score (1 = no assistance to 4 = caesarian section) was assigned at parturition (Burfening et al., 1978). Calf birth BW, sex, and birth date were also recorded. A CE score of 2 or greater was considered as dystocia. Heifers were removed from the herd for reproductive failure, calf death, or injury after calving.

Primiparous Heifers

Two-yr-old primiparous heifers used in this phase of the study and additional primiparous heifers (n = 135) were utilized in a continuation of this study to evaluate supplementation effects during their second breeding season. Primiparous heifers were blocked by previous treatment and randomly assigned to either NS (n = 67) or SUP (0.91 kg/d, 32% CP, DM, n = 68). Primiparous heifers were synchronized with a single $PGF_{2\alpha}$ injection 5 days after being placed with bulls at a 1:20 bull to heifer ratio for 45 d, beginning approximately August 5. Primiparous heifers were managed as a single herd prior to and after the breeding season, and as separate herds (NS or SUP) throughout the breeding season. During the breeding season, primiparous heifers grazed upland range in addition to any supplementation.

Pregnancy diagnosis of primiparous heifers was conducted via transrectal ultrasonography at weaning in November, a minimum of 45 d following bull removal. Females were removed from the herd at weaning for reproductive failure or injury. Prior to calving, females grazed dormant winter range. Prepartum primiparous heifer BW and BCS was measured prior to an expected calving date of May 15. Percent of cows calving in the first 21 d was calculated similar to heifers. A CE score was assigned at birth also similar to heifers.

Statistical Analysis

Treatment was breeding season supplementation. Year was considered the experimental unit with breeding season treatment as a heifer or primiparous heifer as the main effect. Data were analyzed utilizing the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) Development treatment was included as a covariate in the model statement when analyzing heifer data. The proportions of pubertal, pregnant females, dystocia rates, percent bull calves, and percent calved in the first 21 days of the calving season were analyzed using an odds ratio. A P-value of < 0.05 was considered significant with P-values between 0.05 and 0.10 considered a tendency.

RESULTS AND DISCUSSION

Heifers

Heifers had a similar initial BW ($P = 0.88$, 304 ± 2 kg; Table 1). Pubertal status prior to breeding was similar ($P = 0.96$) between treatments. Throughout the breeding season, ADG tended to be greater ($P = 0.05$) for SUP heifers. At pregnancy diagnosis BW

TABLE 1. Effect of supplementation during the breeding season on heifer ADG, BW, BCS, and pregnancy rate in a May calving herd

	Treatment[1]				*P*-value[2]	
	NS	SUP	SEM	TRT	Yr	T×Y
n	128	129				
BW, kg						
Prebreeding	307	306	3	0.88	< 0.01	0.70
Pregnancy diagnosis	350	356	3	0.10	0.17	0.46
Precalving	392	391	4	0.81	< 0.01	0.54
BCS[3]						
Pregnancy diagnosis	5.8	5.8	0.03	0.54	< 0.01	0.13
Precalving	5.2	5.2	0.04	0.28	< 0.01	0.13
ADG, kg/d						
Prebreeding to pregnancy diagnosis	0.43	0.50	0.02	0.05	< 0.01	0.65
Pregnancy diagnosis to precalving	0.23	0.19	0.01	0.16	< 0.01	0.69
Pubertal,[4] %	67	67	4	0.96	< 0.01	0.59
Pregnancy rate, %	68	72	4	0.51	0.38	0.27
Calved in first 21 days, %	71	82	5	0.12	0.64	0.12
Dystocia,[5] %	11	13	5	0.79	0.04	0.05

[1]Heifers grazing upland range were offered either no supplement (NS) or the equivalent of 0.45 kg/hd 32% CP, DM; SUP) supplement delivered 3 times/wk on a pasture basis (35.6 ha) from July 22 to September 5.

[2]TRT: Breeding season treatment main effect, Yr: Year main effect, T×Y: Breeding season treatment by year interactions.

[3]Body condition score (1 = emaciated to 9 = obese; Wagner et al., 1988).

[4]Considered pubertal if blood serum progesterone concentration > 1 ng/ml.

[5]Percentage of females with a calving ease score of 2 or greater (1 = no assistance to 4 = caesarian section; Burfening et al., 1978).

TABLE 2. Effect of supplementation during the breeding season on primiparous heifer ADG, BW, BCS, and pregnancy rate in a May calving herd

	Treatment[1]				*P*-value[2]	
	NS	SUP	SEM	TRT	Yr	T×Y
n	67	68				
BW, kg						
Prebreeding[3]	385	388	5	0.73	< 0.01	0.12
Pregnancy diagnosis	376	397	5	< 0.01	0.09	0.68
Precalving	430	434	6	0.60	< 0.01	0.37
Prebreeding[4]	446	458	7	0.19	< 0.01	0.36
BCS[5]						
Prebreeding[3]	5.3	5.3	0.05	0.89	0.69	0.20
Pregnancy diagnosis	5.0	5.3	0.06	< 0.01	0.29	0.62
Precalving	5.0	5.2	0.07	0.09	< 0.01	0.37
Prebreeding[4]	5.7	5.6	0.07	0.57	0.02	0.82
ADG, kg/d						
Precalving to prebreeding	0.01	0.02	0.02	0.81	< 0.01	0.06
Prebreeding to pregnancy diagnosis	- 0.07	0.08	0.02	< 0.01	< 0.01	0.03
Pregnancy diagnosis to precalving	0.40	0.30	0.03	< 0.01	< 0.01	0.73
Precalving to prebreeding	0.17	0.26	0.05	0.18	0.69	0.98
Pregnancy rate, %	75	81	6	0.41	0.93	0.52
Calved in first 21 days, %	84	83	6	0.91	0.56	0.52
Dystocia,[6] %	0	0	31	0.99	1.0	1.0

[1]Primiparous heifers grazing upland range were offered either no supplement (NS) or the equivalent of 0.91 kg/hd (32% CP, DM; SUP) supplement delivered 3 times/wk on a pasture basis (35.6 ha) from August 5 to September 19.

[2]TRT: Breeding season treatment main effect, Yr: Year main effect, T×Y: Breeding season treatment by year interactions.

[3]BW and BCS recorded preceding the breeding season as a primiparous heifer.

[4]BW and BCS recorded preceding the breeding season as a 3-yr-old cow.

[5]Body condition score (1 = emaciated to 9 = obese; Wagner et al., 1988).

[6]Percentage of females with a calving ease score of 2 or greater (1 = no assistance to 4 = caesarian section; Burfening et al., 1978).

also tended ($P = 0.10$) to be greater for SUP heifers, but similar ($P = 0.81$) prior to calving. Heifer BCS was similar ($P \geq 0.28$) between treatments at both time points. Percent of pregnant heifers was similar ($P = 0.51$) between treatments. Overwinter, heifers in both treatments had similar ($P = 0.16$) ADG. Percentage of heifers calving in the first 21 d of calving season was also similar ($P = 0.12$). Dystocia rates were similar ($P = 0.79$) between treatment groups.

Primiparous Heifers

Primiparous heifers had a similar initial BW ($P = 0.73$, 387 ± 3 kg; Table 2). Supplemented primiparous dams had a greater BW and BCS ($P < 0.01$) at pregnancy diagnosis as NS primiparous heifers had a decrease in BW and BCS during the breeding season. Linden et al. (2014) suggests this decline in BW and BCS, despite an increase in DMI, is a byproduct of the primiparous heifer's inability to consume enough low-quality forage during early lactation to meet the demands of growth and lactation. Pregnancy rates were not affected (P = 0.41) by breeding season treatment. This increase in BW at pregnancy diagnosis corresponds with a greater ADG ($P < 0.01$) throughout the breeding season in SUP cows. Prior to calving and at subsequent prebreeding, treatment groups had similar BW and BCS ($P \geq 0.09$). Percentage of cows calving in the first 21 d was similar ($P = 0.91$) between treatments. Dystocia rates for SUP and NS primiparous heifers was similar ($P = 0.99$). Overwinter, NS cow ADG was greater ($P < 0.01$), but did not differ ($P = 0.18$) between treatments from precalving as a 2-yr-old primiparous heifer to prebreeding as a 3-yr-old cow.

Requirements for growing heifer calves (9% CP and 58% TDN, DM) are less than lactating primiparous heifers (12.8% CP and 66% TDN, DM; NRC,

TABLE 3. Pregnancy rates in a March and May calving herd and associated forage quality during the breeding season in the Nebraska Sandhills

	Pregnancy rate, %					TDN, %		CP, %	
			May[2]						
	Mar[1]	n[3]	NS	SUP	SEM[4]	Jun[5]	Aug[6]	Jun[5]	Aug[6]
Heifer									
2012	85	66	59	82	9	62	59	10	9
2013	84	65	66	58	9	80	68	19	12
2014	84	68	71	70	8	62	60	14	10
2015	82	58	76	76	8	56	58	12	8
Primiparous Heifer									
2013	84	62	80	75	8	80	68	19	12
2014	65	34	71	82	11	62	60	14	10
2015	87	39	74	85	10	56	58	12	8

[1]Heifers (n = 548) and primiparous heifers (n = 176) as part of a March calving herd grazing upland range.

[2]Heifers and primiparous heifers as part of a May herd grazed upland range were offered either no supplement (NS) or a 32% CP (DM; SUP) supplement delivered 3 times/wk on a pasture basis throughout a 45 d breeding season. Heifers received 0.45 kg/hd (July 22 to September 5) and primiparous heifers received 0.91 kg/hd supplement (August 5 to September 19).

[3]Number of NS and SUP heifers in a May calving herd by year.

[4]Maximal SEM of May calving NS vs. SUP heifers.

[5]March calving herd breeding season represented by a forage sample taken in June.

[6]May calving herd breeding season represented by the average of forage of samples taken in July and September.

TABLE 4. Effects of breeding season treatment on calf BW and ADG in a May calving herd

	Treatment[1]			*P*-value[2]		
	NS	SUP	SEM	TRT	Yr	T×Y
First Calf[3]						
Birth BW, kg	29	30	0.5	0.05	< 0.01	< 0.01
Prebreeding BW, kg	95	95	3	0.95	< 0.01	0.76
Weaning BW, kg	166	177	3	< 0.01	< 0.01	0.62
Birth to prebreeding, kg/d	0.85	0.87	0.01	0.36	< 0.01	0.29
Prebreeding to weaning, kg/d4	0.64	0.73	0.01	< 0.01	< 0.01	0.58
Second Calf[4]						
Birth BW, kg	36	34	0.7	0.17	0.02	0.44
Prebreeding BW, kg	93	96	2	0.36	< 0.01	0.43
Weaning BW, kg	185	190	6	0.47	< 0.01	0.91
Birth to prebreeding, kg/d	1.0	1.1	0.03	0.45	< 0.01	0.12
Prebreeding to weaning, kg/d	0.58	0.61	0.03	0.57	< 0.01	0.96

[1]Heifers and primiparous heifers grazing upland range were offered either no supplement (NS) or a 32% CP (DM) supplement (SUP) delivered 3 times/wk on a pasture basis (35.6 ha) for a 45 d breeding season. Heifers received 0.45 kg/hd supplement (beg. July 22), and primiparous heifers received 0.91 kg/hd supplement (beg. August 5).

[2]TRT: Breeding season treatment as a primiparous heifer main effect, Yr: Year main effect, T×Y: Breeding season treatment by year interactions.

[3]Calf nursing NS or SUP primiparous heifer.

[4]Calf nursing previously supplemented NS or SUP cow.

2000). It is interesting to note the variability in pregnancy response to supplementation and is most dramatic when forage conditions are below requirements (Table 3). In nearly every year, March-born heifer and primiparous heifer pregnancy rates were greater than May-born, which coincides with a greater breeding season forage quality.

Calf Performance

First calf birth BW tended ($P = 0.05$; Table 4) to be greater for SUP dams. First calf prebreeding BW was similar ($P = 0.95$) between heifer treatments. Average daily gain from birth to prebreeding was also similar ($P = 0.36$) between calves born to SUP vs. NS heifers. At weaning, calves nursing SUP dams had a greater BW ($P < 0.01$) and gained 0.09 kg/d greater ($P < 0.01$) throughout the breeding season than NS counterparts. The increase in first calf weaning BW and ADG, without affecting dam BW or BCS, is likely due to calves consuming supplement directly, rather than nutrient partitioning by the dam. Edwards et al. (2017), reported milk production had no influence on calf BW at weaning for dams with abundant feed resources. Tedeschi and Fox (2009), suggest an inverse relationship between milk consumption and feed intake. Stalker et al. (2006) reported greater weaning BW in calves provided higher quality forage in the form of subirrigated meadow in a March calving herd and found no difference in pregnancy rates of mature cows.

Second calf birth BW and calf sex were similar (P □ 0.17; Table 4) by breeding season treatment as a primiparous heifer. Additionally, second calf prebreeding and weaning BW were similar (P □ 0.36) among dam's prior treatment during the breeding season. Correspondingly, second calf ADG from birth to prebreeding and prebreeding to weaning did not differ (P □ 0.45) between treatment groups.

IMPLICATIONS

Supplementation prior to and throughout the breeding season numerically increased pregnancy rates in both heifers and primiparous heifers in a May calving herd. Supplemented heifer calves tended and primiparous heifers had greater BW at pregnancy diagnosis despite no significant effect on pregnancy rates. Calves nursing supplemented dams had greater weaning BW. It is possible greater supplementation may be needed to illicit a significant effect on pregnancy rate.

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The effects of shredded sugar beets on sheep nutrient metabolism and ruminal characteristics[1]

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ABSTRACT: Non-harvested sugar beets represent an underutilized yet cost effective feedstuff for livestock producers in Montana. The objective of this study is to evaluate the effects of shredded sugar beets fed at increasing levels on sheep nutrient metabolism. Eight wethers were used in a 4 × 4 replicated Latin Square and allocated to one of four dietary treatments where sugar beets replaced 0% (0SB), 15% (15SB), 30% (30SB), or 45% (45SB) of barley on a DM basis. The MIXED procedure of SAS was used for statistical analysis. The digestibility of DM, NDF, ADF, and nitrogen were not affected ($P \geq 0.10$) by treatment. There was quadratic tendency observed for ADF digestibility ($P = 0.10$), with 0SB and 45SB wethers being greater than 15SB and 30SB wethers. Nitrogen concentration in fecal matter and serum were not affected by treatment ($P \geq 0.22$). Ruminal ammonia and acetate concentrations showed a linear tendency ($P \leq 0.09$) to increase with increasing sugar beets in the diet. Propionate concentration decreased linearly ($P = 0.05$) with increasing sugar beets in the diet. The acetate to propionate ratio increased linearly ($P = 0.03$) with increasing sugar beets in the diet. Butyrate concentration demonstrated a treatment × time effect ($P = 0.01$), where butyrate concentration decreased with increasing sugar beets in the diet at 0700, but increased with increasing sugar beets in the diet at 1300. Valerate, isobutyrate, and isovalerate concentrations were not affected by treatment ($P \geq 0.25$). Ruminal pH was not affected ($P \geq 0.39$) by treatment at 0700, but a quadratic effect was observed for pH at 1300 ($P = 0.05$), with 15SB having the greatest pH (6.99) and 45SB having the least pH (6.51). Based on the results of our previous study and the results of the current study, we conclude that sugar beets can replace barley up to 45% in the diet and have no deleterious effects on nutrient metabolism.

Key words: nutrient metabolism, ruminal characteristics, sheep, sugar beets
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INTRODUCTION

Montana is a major producer of sugar beets in the Northern Great Plains (5th in the U.S.; USDA, 2015a), and excess or non-harvested sugar beets could provide a readily available alternative feedstuff for cattle and sheep producers. In Montana, during the 2014-2015 sugar beet harvest, approximately 45.2 million pounds of sugar beets were not harvested (USDA, 2015b).

Sugar beets are an excellent energy source (81% TDN; Lardy and Schafer, 2008), which can be complimentary to, or even replace traditional feedstuffs, such as barley or corn. Sugar beets differ due to the much higher moisture content (70-80% moisture; Lardy and Schafer, 2008) and how they store energy in the form of sugar rather than starch (12-20% sugar; Agribusiness Handbook, 2009). Numerous studies have observed the effects of sucrose on nutrient metabolism and the rumen environment (Broderick and Smith, 2001; Vallimont et al., 2004). Some studies have observed an increase in NDF digestibility when sugar beets replaced a high starch feed source (Huhtanen, 1988; Arrizon et al., 2012). Because rumen anaerobic fungi are capable of fermenting sugars such as sucrose and glucose, the sugar from sugar beets may be able to create a favorable rumen environment resulting in enhanced NDF digestibility (Emanuele, 2004).

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Therefore, the objective of this study is to evaluate the effects of shredded sugar beets fed at increasing levels on sheep nutrient metabolism. We previously conducted a study that suggests sugar beets can replace barley up to 45% in the diet on a DM basis when fed to backgrounding steers (McGregor et al., 2016) without deleterious effects on performance. Based on the previous data, we hypothesize that when sheep are fed increasing levels of sugar beets (0, 15, 30, and 45% of DM), there will be no deleterious effects on fiber or nitrogen digestibility.

MATERIALS AND METHODS

All procedures were approved by the animal care and use committee of Montana State University (#2016-AA09).

A 4 × 4 replicated Latin Square design was used to evaluate the effects of four diets varying in sugar beet concentration on the nutrient metabolism of wethers. Dietary treatments (Table 1) were; 1) 0% sugar beets (**0SB**), 2) 15% sugar beets (**15SB**), 3) 30% sugar beets (**30SB**), and 4) 45% sugar beets (**45SB**). Sugar beets directly replaced barley on a DM basis. All dietary treatments were formulated to meet or exceed the nutrient requirements of growing wethers (NRC, 2007). Each experimental period was 20 d in length with 4 d between periods (d 1 to 5; to remove wethers from metabolism crates). All wethers were kept in a single pen with ad libitum access to hay and water d 1 to 5. On d 5 wethers were assigned to a dietary treatment and placed in metabolism crates in a temperature controlled enclosed room for a 10-d adaptation period to metabolism crates and diets. These wethers were on a 12 h light, 12 h dark schedule. Each treatment was fed as a TMR at 3% of each wethers initial BW (as fed).

Total mixed ration (TMR) samples were collected d 15 through d 19 and ort samples were collected d 16 through d 20. Ort and TMR samples were dried in a 60°C forced air drying oven for 48-h for DM analysis. Total fecal output was collected and weighed on d 16 through d 20 with 7.5% of the total fecal sample collected, weighed, and placed in a 60°C forced air drying oven for 96-h for DM analysis. On d 16 through d 20, total urine output was collected. Sufficient 6 *N* HCl (100 mL) was added daily to urinals to maintain urine pH < 3. A 25% subsample of the total urine weight was collected and composited by individual lamb.

Blood samples were collected on d 15 through d 19, 4 hours post-prandially via jugular venipuncture into 16 × 100 mm blood collection tubes (no. 367988; BD Vacutainer, Franklin Lakes, NJ) and refrigerated (4°C) for 2.5 h. Blood samples were centrifuged at 2500 × *g* for 20 minutes at 4°C. Serum was collected into 5-mL polypropylene tubes. Samples were then stored at -20°C until analysis.

TABLE 1. Ingredient and nutritional composition of diets fed to growing wethers (DM basis)

	Dietary Treatment[1]			
Item	0SB	15SB	30SB	45SB
Ingredient, %				
Sugar beets[2]	—	15.00	30.00	45.00
MSU barley	45.00	30.00	15.00	—
Grass hay	46.00	41.00	36.90	32.80
Soybean meal	5.50	10.40	14.80	19.00
NaCl	0.25	0.25	0.25	0.25
Decoquinate	1.35	1.35	1.35	1.35
Calcium carbonate	1.00	1.10	0.85	0.75
Mineral premix	0.90	0.90	0.90	0.90
Nutritional Composition[4]				
DM, %	28.33	24.97	22.59	20.72
TDN, %	66.80	65.80	64.80	63.60
CP, %	15.80	15.70	15.40	15.10
Ca:P	2.30	2.50	2.40	2.47

[1]Diets (DM basis) were formulated for growing wethers according to NRC (2007). Treatments: 0SB) 0% sugar beets, 15SB) 15% sugar beets, 30SB) 30% sugar beets, & 45SB) 45% sugar beets.

[2]Sugar beets were coarse ground with a flail chopper designed for woody biomass, to reduce choking hazard.

Rumen fluid was extracted from all sheep on d 19 of each period at 0700 (pre-prandial) and at approximately 1300 (post-prandial). Rumen fluid was obtained by inserting a clear vinyl tube into the rumen of each wether through the esophagus. Rumen fluid was pulled through the tube using an air-tight syringe on the opposite end of the tube. Rumen fluid pH measurements were taken directly after extraction, then samples were stored at -20°C.

Laboratory Analysis

Total mixed ration, ort, and fecal samples were ground to pass a 1 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ort samples were composited by lamb within period and TMR samples were composited by period. Feed, orts, and fecal samples were analyzed for NDF (method 2002.04; AOAC, 2005) and ADF (method 973.18; AOAC, 2005) by using an Ankom 2000 Fiber Analyzer (Ankom Co., Fairport, NY). Alpha-amylase and sodium sulfite were used in the NDF procedure. Nitrogen concentrations were also measured (method 2001.11; AOAC, 2010).

Serum urea nitrogen (SUN) concentrations were determined by using a commercial colorimetric kit (Teco Diagnostics, Anaheim, CA) with intra- and interassay CV less than 12%.

TABLE 2. Nutrient metabolism characteristics of growing wethers fed increasing concentrations of sugar beets in the diet

	Dietary Treatment[1]					Orthogonal Contrasts[3]		
Item	0SB	15SB	30SB	45SB	SEM[2]	Linear	Quadratic	Cubic
Initial BW, kg	36.65	36.65	36.65	36.65	0.00	1.00	1.00	1.00
Daily DMI, g/kg BW	29.12	26.01	28.03	30.43	2.51	0.61	0.30	0.68
Daily NDF intake, g/kg BW	12.48	9.57	10.50	11.14	0.85	0.41	0.04	0.27
Daily ADF intake, g/kg BW	7.80	5.96	6.88	7.13	0.60	0.68	0.09	0.20
Daily nitrogen intake, g/kg BW	0.68	0.63	0.90	0.74	0.07	0.10	0.33	0.02
Total tract digestibility, %								
DM	70.34	67.23	71.29	71.4	0.20	0.44	0.44	0.25
NDF	57.07	50.70	52.71	53.41	35.02	0.57	0.33	0.54
ADF	51.64	40.41	48.64	50.58	39.11	0.77	0.10	0.14
Nitrogen	74.83	72.66	74.95	72.22	1.88	0.37	0.85	0.19
Daily nitrogen excretion, g/kg BW								
Fecal	0.18	0.17	0.17	0.21	0.02	0.33	0.29	0.93
Serum urea nitrogen, mg/dL	5.65	5.03	4.39	4.87	0.53	0.22	0.31	0.63

[1]Maternal diets (DM basis) were formulated for growing wethers according to NRC (2007). Treatments: 0SB) 0% sugar beets, 15SB) 15% sugar beets, 30SB) 30% sugar beets, & 45SB) 45% sugar beets.

[2]Greatest SEM presented (n = 8).

[3]*P*-value for linear, quadratic, and cubic effects of increasing sugar beet concentration in the diet.

Rumen samples were analyzed for ammonia concentration (RAN) using methods similar to those described by Sigma Technical Bulletin #640 (Sigma Diagnostics, St. Louis, MO), Chaney and Marback (1962), Horn and Squire (1967), and Weichselbaum et al., (1969). This procedure is based on the premise that ammonia will react with hypochlorite and phenol in the presence of sodium nitroprusside, which will yield indophenol. Ammonia concentration is directly proportional to the absorbance of indophenol, which was read using a spectrophotometer at 570 nm.

Rumen samples were also analyzed for individual VFA concentrations using a gas chromatography procedure similar to that described by Baumgardt (1964), Supleco Inc. bulletin 749E (Supleco Inc.,1975), Byers (1979), and Fritz and Schenk (1979).

Statistical Analysis

Data were analyzed as a replicated Latin Square, with lamb serving as the experimental unit. Nutrient metabolism data were analyzed using the MIXED procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC). The model included the fixed effects of dietary treatment, period, and replicate. The period and dietary treatment interaction and replicate and treatment interaction served as random effects. Day served as the repeated measure used to analyze daily DMI and SUN concentrations using the variance components covariance structure, selected due to the lowest Akaike's information criteria. Fixed effects for SUN and daily DMI were dietary treatment, day, and the interaction. Rumen fluid analysis was conducted with the fixed effects of dietary treatment, time of collection, and the interaction. Time of collection served as the repeated measure with the variance component covariance structure. Linear, quadratic, and cubic orthogonal contrasts of sugar beet inclusion rate served to partition dietary treatment effects. Significance was set at $P \leq 0.05$, with tendencies set at $P \leq 0.10$.

RESULTS AND DISCUSSION

The nutrient metabolism results are presented in Table 2. Initial body weights, by design, did not differ ($P = 1.00$) between treatments. Daily DMI was not affected by treatment ($P \geq 0.30$). The intake of NDF was affected quadratically by treatment ($P = 0.04$), with the greatest being 0SB and the least being 15SB wethers. The intake of ADF demonstrated a quadratic tendency ($P = 0.09$), with the greatest being for 45SB and the least being 15SB. These results contrast with Huhtanen (1988), where both NDF intake and ADF intake were greater when sugar beet pulp replaced barley in a silage based diet for cattle. There was a cubic effect for daily nitrogen intake ($P = 0.02$) where nitrogen intake was greatest in the 30SB wethers and 15SB had the least nitrogen intake.

Dry matter digestibility was not affected by treatment ($P \geq 0.25$). Results from the current study agree with Vallimont et al. (2004) that reported no effects on DM digestibility when corn starch was replaced with sucrose in vitro. In contrast, Huhtanen (1988) demon-

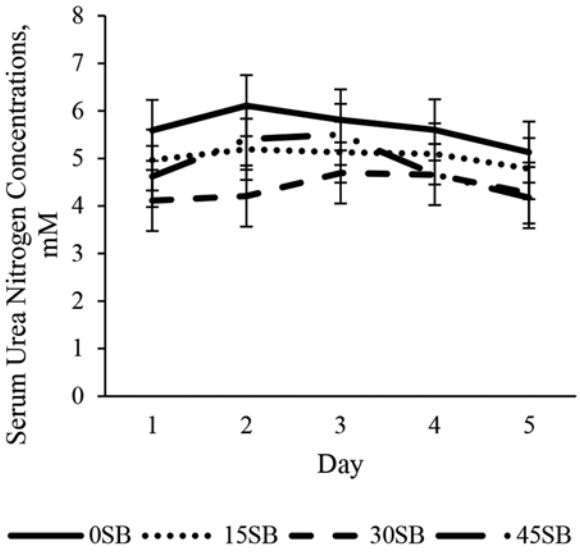

Figure 1. Effects of increasing sugar beet concentrations on serum urea nitrogen concentrations. Treatment x day: $P = 0.95$; Day: $P = 0.11$; and treatment: $P = 0.42$.

strated a decrease in DM digestibility when sugar beet pulp replaced barley in a silage based diet.

There was no treatment effect on NDF digestibility ($P \geq 0.33$), however there was a quadratic tendency for ADF digestibility ($P = 0.10$) with the lowest value for 15SB (40.41%) and the highest value for 0SB (51.64%). Our results differ from those observed by Arrizon et al. (2012) that reported increased NDF digestibility when dried shredded sugar beets replaced steam flaked corn. The results of the current study also differ from results observed by Huhtanen (1988) who observed an increase in NDF and ADF digestibility when sugar beet pulp replaced barley in a silage based diet.

Nitrogen digestibility, fecal nitrogen excretion, and SUN were not affected by treatment ($P \geq 0.19$). There was no treatment × day interaction ($P = 0.95$; Figure 1) for SUN concentrations. These results agree with the results from Vallimont et al., (2004) where replacing corn starch with sucrose in vitro also had no effect on nitrogen digestibility.

Rumen fluid characteristics are presented in Table 3. There was a linear tendency ($P = 0.07$) for RAN concentrations to increase as sugar beets increased in the diet. This result is divergent to results provided by Broderick and Smith (2001), who observed a quadratic response to increasing sugar in the diet. In addition, our results also differ from Huhtanen (1988) and Rooke et al., (1992) where a decrease in RAN occurred when sugar beet pulp or molasses sugar beet feed replaced barley in a silage based diet.

There was a linear tendency ($P = 0.09$) for acetate concentrations to increase with increasing sugar beet concentrations in the diet. This is in agreement with results provided by Voelker and Allen (2003) and Arrizon et al., (2012) where an increase in acetate occurred when beet pulp or dried shredded sugar beets replaced high-moisture corn or steam flaked corn, respectively

Propionate concentrations decreased linearly ($P = 0.05$) as sugar beets increased in the diet. This result is similar to Voelker and Allen (2003) and Arrizon et al., (2012) who also observed a decrease in propionate concentrations when beet pulp or dried shredded sugar beets replaced high-moisture corn or steam flaked corn, respectively. However, Huhtanen (1988) observed an increase in propionate concentrations when sugar beets replaced barley in a silage based diet.

As sugar beets increased in the diet, the acetate to propionate ratio increased linearly ($P = 0.03$). This result is similar to Voelker and Allen (2003) when they observed an increase in the acetate to propionate ratio when beet pulp replaced high moisture corn. In addition, Vallimont et al. (2004) observed a quadratic effect on the acetate to propionate ratio when corn starch was replaced with sucrose. A greater acetate to propionate ratio is indicative of a fiber based diet, and a low acetate to propionate ratio is indicative of a concentrate based diet, as seen by Lana et al., (1998).

There was a treatment × time effect ($P = 0.01$) for butyrate where butyrate concentrations declined as sugar beet concentrations increased in the diet (preprandially), then increased as sugar beets increased in the diet 4 h (post-prandially). This is similar with Huhtanen (1988) and Rooke et al., (1992) where an increase in butyrate concentrations were observed when sugar beet pulp or molasses sugar beet feed replaced barley in silage based diets. This also is similar to the results provided by Voelker and Allen (2003) and Arrizon et al., (2012) where an increase in butyrate concentrations was observed when beet pulp or dried shredded sugar beets replaced high-moisture corn or steam flaked corn, respectively.

Valerate, isobutyrate, and isovalerate were not affected by treatment ($P \geq 0.25$). These results are similar to Arrizon et al., (2012) where no treatment effect on isobutyrate or isovalerate concentrations was observed when dried shredded sugar beets replaced steam flaked corn. However, Arrizon et al., (2012) did observe an increase in valerate when dried shredded sugar beets replaced steam flaked corn.

Ruminal pH at 0700 was not affected by treatment ($P \geq 0.39$). This result is similar to those observed by Huhtanen (1988) and Arrizon et al., (2012) where no treatment effect was observed for pH when sugar beet pulp or dried shredded sugar beets replaced barley or steam flaked corn, respectively. However, dietary treatment imposed a quadratic effect on pH at 1300 as sugar beets increased in the diet ($P = 0.05$).

TABLE 3. Ruminal characteristics of growing wethers fed increasing concentrations of sugar beets in the diet.

	Dietary Treatment[1]						Orthogonal Contrasts[3]		
Item	0SB	15SB	30SB	45SB	SEM	*P*-value[2]	Linear	Quadratic	Cubic
Ammonia, mg/dL[4]					2.81	0.59	0.07	0.14	0.27
0700	22.61	22.27	24.86	26.37					
1300	27.19	20.80	27.49	31.35					
VFA, mol/100 mol[4]									
Acetate					3.12	0.85	0.09	0.60	0.89
0700	79.68	81.00	83.35	85.13					
1300	76.90	81.38	83.71	82.54					
Propionate					3.16	0.35	0.05	0.90	0.85
0700	23.93	23.65	20.55	20.03					
1300	29.73	25.22	21.82	18.87					
Butyrate					2.20	0.01	0.62	0.67	0.80
0700	15.03	13.21	13.49	11.13					
1300	15.33	15.49	17.37	21.39					
Valerate					0.24	0.43	0.26	0.63	0.51
0700	1.48	1.18	1.50	1.40					
1300	1.54	1.27	1.09	0.97					
Isobutyrate					3.00	0.36	0.39	0.95	0.45
0700	2.17	2.66	2.63	3.00					
1300	0.75	0.75	0.50	0.56					
Isovalerate					3.82	0.34	0.25	0.85	0.52
0700	2.72	3.31	3.48	3.83					
1300	0.75	0.89	0.52	0.68					
A:P ratio[5]					0.64	0.14	0.03	0.90	0.92
0700	3.74	4.17	4.43	4.66					
1300	2.83	3.52	4.27	5.42					
pH[3]					0.15	0.75	0.10	0.08	0.39
0700	7.45	7.59	7.32	7.29					
1300	6.73	6.99	6.84	6.51					

[1]Maternal diets (DM basis) were formulated for growing wethers according to NRC (2007). Treatments: 0SB) 0% sugar beets, 15SB) 15% sugar beets, 30SB) 30% sugar beets, & 45SB) 45% sugar beets.

[2]*P*-value for the treatment x time interaction.

[3]*P*-value for linear, quadratic, and cubic effects of increasing sugar beet concentration in the diet.

[4]Rumen fluid extraction and pH measurements took place at 0700 and at 1300 on the last day of each period.

[5]A:P ratio = Acetate to propionate ratio.

Measurements of pH at 1300 was greatest for 15SB and was least for 45SB.

IMPLICATIONS

The current research suggests that whole shredded sugar beets can replace barley up to 45% without having any deleterious effects on fiber or nitrogen digestion. Findings suggest shredded sugar beets result in a different rumen environment compared to energy sources with high starch content where butyrate concentrations as well as the acetate to propionate ratio increases, while propionate decreases with increasing sugar beets in the diet. Utilizing sugar beets may potentially provide greater economic returns for sugar beet producers, as well as decreasing feed costs for livestock producers.

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Metabolic regulation by stress systems in bovine myoblasts and ovine fetal skeletal muscle

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ABSTRACT: tress factors including catecholamines and inflammatory cytokines are known to regulate glucose metabolism. However, the underlying mechanisms and the impact on developing muscle are not fully understood. Therefore, the objective of this study was to determine how β adrenergic agonists and inflammatory cytokines alter glucose metabolic homeostasis in myoblasts and fetal skeletal muscle fibers. In Experiment 1, glucose uptake and glucose oxidation rates were determined in primary bovine myoblasts (muscle stem cells) incubated for 4 days in differentiation media with or without insulin, and/or β_1 adrenergic agonist (Ractopamine HCl), or β_2 adrenergic agonists (Zilpaterol HCl). In Experiment 2, pregnant ewes were treated with saline (controls) or bacterial endotoxin (**LPS**) from day 100-115 of gestation (term = 150 days). On gestational day 122, ewes were euthanized and fetal soleus muscle was isolated tendon-to-tendon and split longitudinally. To determine glucose uptake and glucose oxidation rates, soleus strips were incubated in KHB that was un-spiked (basal) or spiked with either insulin or TNFα. In differentiated bovine myoblast cultures, insulin increased ($P < 0.05$) glucose uptake and oxidation when compared to basal. β_2 adrenergic agonist likewise increased ($P < 0.05$) glucose uptake and oxidation from basal to levels that did not differ from insulin alone. β_1 adrenergic agonist did not change glucose uptake for basal levels and only slightly increased ($P < 0.05$) glucose oxidation. Moreover, when β_1 adrenergic agonist was added in combination with insulin, glucose uptake and oxidation were both less ($P < 0.05$) than with insulin alone. Interestingly, when β_2 adrenergic agonist was added in combination with insulin, glucose uptake was not different from insulin alone but glucose oxidation was decreased ($P < 0.05$). In primary ovine fetal soleus muscle, glucose uptake was not different between control and LPS fetuses under basal, insulin-stimulated, or TNFα-stimulated conditions. However, insulin-stimulated glucose oxidation and TNFα-stimulated glucose oxidation were both greatly decreased ($P < 0.05$) in LPS fetal muscle compared to controls. Our findings show that adrenergic and inflammatory mediators have insulin dependent and insulin independent effects on glucose metabolism and that regulation by these stress systems is present in developmental stages, as demonstrated by responses in both stem cells and fetal muscle.

Key words: β agonist, metabolic syndrome, pro-inflammatory cytokines
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INTRODUCTION

Factors that activate stress systems, including inflammatory cytokines and β adrenergic agonists, are known to impact metabolism in both myoblasts and skeletal muscle fibers. Skeletal muscle accounts for more than 80% of insulin-stimulated glucose metabolism (DeFronzo et al., 1981) and therefore plays a critical role in glucose homeostasis. Inflammatory cytokines such as TNFα can acutely enhance glucose metabolism despite antagonizing insulin signaling (Cadaret et al., 2016). However, we speculate that chronic exposure of developing muscle to inflammatory cytokines may reduce sensitivity to them and thus dampen their direct capacity to stimulate glucose oxidation. Inflammation is known to cause insulin resistance (Marette et al., 2014), and thus fetuses that are chronically exposed to inflammatory cytokines during late gestation are likely at a greater risk for developing metabolic dysfunction from the combination of insulin resistance and reduced sensitivity to cytokines (Pickup and Crook, 1998; Spranger et al., 2003).

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The β adrenergic agonists, ractopamine HCl (β_1) and zilpaterol HCl (β_2), enhance muscle growth efficiency and decrease fat accumulation in finishing cattle when used as feed additives (Mersmann, 1998), although the mechanisms underlying this increased efficiency are not fully understood. Due to our growing population, increased beef consumption, and greater competition for available land, producers benefit from using these products. The use of β adrenergic agonists yields more meat from the same number or even fewer animals (Strydom, 2016), thus benefiting the US economy (Centner et al., 2014). Our objectives for this study were to determine the effects of sustained inflammation on the ability of inflammatory cytokines to regulate insulin-stimulated glucose metabolism in fetal skeletal muscle and to determine whether β adrenergic agonists have similar effects on glucose metabolism in bovine myoblasts (muscle stem cells) as previously found in mature muscle.

MATERIALS AND METHODS

Animals and Treatments

The following experiments were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Studies were conducted at the University of Nebraska-Lincoln Animal Science Complex, which is accredited by the American Association for Accreditation of Laboratory Animal Care.

Experiment 1. Primary myoblasts were isolated as previously described (Yates et al., 2014) from four Angus-cross steers slaughtered at one year of age. Isolated myoblasts were seeded onto 12-well tissue culture plates to measure glucose uptake or 6-well tissue culture plates to measure glucose oxidation at an initial seeding density of 20,000 cells per well for both plates. All plates were coated with poly-L-lysine and bovine fibronectin. Cells were incubated in complete growth media (DMEM, 20% FBS, 1% Ab/Am, 0.4% Fungizone, and 0.5% Gentamicin) for 24 hours then incubated in treatment-spiked differentiation media (DMEM, 2% FBS, 1% Ab/Am, 0.4% Fungizone, and 0.5% Gentamicin) for four days. Differentiation media was spiked with one of the following treatments: no additive (basal), insulin (5 mU/ml Humulin-R), β1 agonist (1 μM ractopamine HCl), β2 agonist (0.05 μM zilpaterol HCl), β1 agonist + insulin, or β2 agonist + insulin. All additives were purchased from Sigma-Aldrich (St. Louis, MO) with the exception of Humulin-R (Eli Lilly, Indianapolis, IN)

To determine glucose uptake, cells were pre-incubated at 37ºC for 1 hour in treatment-spiked Krebs-Henseleit Buffer (KHB) media containing 0.1% bovine serum albumin (BSA), 5mM glucose, and 35 mM mannitol. Cells were then washed at 37ºC for 20 minutes in treatment-spiked glucose-free KHB media with 0.1% BSA and 40 mM mannitol. Cells were then incubated at 37º C for 20 minutes in treatment-spiked KHB media with 1 mM [^{3}H] 2-deoxyglucose (300 μCi/mmol) and 39 mM [U-^{14}C] mannitol (12.5 μCi/mmol), and 0.1% BSA. Media was then removed and cells were thrice washed with ice-cold PBS and nuclear stained with 5 μg/ml Hoeshst 3342 (Thermo-Fischer). Cells were imaged and cell number was estimated by counting with Olympus CellSense Software. Cells were then lysed with 2 mM NaOH and lysates were transferred to scintillation vials and mixed with 20 ml UltimaGold scintillation fluid. Specific activities of [^{3}H] to determine glucose uptake and [^{14}C] to estimate extracellular fluid volume were measured using a Beckman-Coulter 1900 TA LC counter (Brea, CA). Specific activity of media was estimated from 10-μl aliquots mixed with 500-μl double-distilled water. All radioactive materials and scintillation fluids were purchased from Perkin-Elmer (Waltham, MA). Four technical replications per condition were performed and averaged for each animal.

Before measuring glucose oxidation rates, cell populations in each well were imaged using Olympus CellSense Software. Three images per well were taken using bright field and used to estimate the number of cells per well. Cells were then pre-incubated at 37ºC for 1 hour in treatment-spiked KHB with 0.1% BSA and 5 mM glucose and washed at 37ºC for 20 minutes in glucose-free treatment-spiked KHB with 0.1% BSA. To determine glucose oxidation, cells were sealed in dual-well chambers using a rubber gasket and incubated at 37º C for 120-min. in treatment-spiked gassed (95% O_2: 5% CO_2) KHB media and containing 5 mM [^{14}C-U] D-glucose (2.5 μCi/mmol) and 0.1% BSA. In the adjacent well, 2 mM NaOH was injected to capture CO_2 produced. After the incubation period, plates were cooled for 2 minutes at -20º C and 2M HCl was injected into the media through the rubber seal. Plates were then incubated at 4ºC for 1 hour to release bicarbonate-bound CO_2 from media. Chambers were opened and the NaOH was collected and mixed with 20 ml UltimaGold scintillation fluid in a scintilation vial. Specific activity of $^{14}CO_2$ and media were determined as described above. Background was determined from no-cell control wells with tracer media. Three technical replications per condition were averaged for each animal.

Experiment 2. Timed-mated Poly Pay ewes carrying twins received an IV injection of sterile saline (Controls; n = 8) or 0.1 μg/kg BW of bacterial lipopolysaccharide (LPS; n = 6) from *E. coli O55:B5 (*Sigma-Aldrich, St. Louis, MO*)* every third day from day 100 to 115 of gestation (term = 150 days).

Animals were euthanized at 140 ± 1 days of gestational age and fetal soleus muscles were isolated tendon-to-tendon, washed in ice-cold PBS, and split longitudinally into intact strips (150-400 mg). Strips were pre-incubated and washed in treatment-spiked KHB as described above. For fetal muscle strips, however, medias were spiked with the following treatments: no additive (basal), insulin (5 mU/ml Humulin-R), or TNFα (20 ng/ml hTNFα). All additives were purchased from Sigma-Aldrich (St. Louis, MO) with the exception of Humulin-R (Eli Lilly, Indianapolis, IN). To determine glucose uptake, muscle strips were incubated at 37º C for 20 minutes in treatment-spiked KHB with 1 mM [^{3}H]2-deoxyglucose and 39 mM [^{14}C] mannitol as described above. Afterward, muscle strips were removed, washed thrice in ice-cold PBS, weighed, and lysed in 2 M NaOH at 37º C for 1 hour. Specific activity of ^{3}H and ^{14}C was determine for lysates and media as above.

To determine glucose oxidation, fetal muscle strips were placed in sealed dual-well chambers, pre-incubated for 1h, and washed for 20 minutes in treatment-spiked KHB as described above. Muscle strips were then incubated at 37º C for 120-min. in treatment-spiked gassed KHB with 5 mM [^{14}C-U] D-glucose (.25 μCi/mmol), and 2M NaOH was put into the adjacent wells to capture CO_2 produced. After the 120-min. incubation, the chambers were cooled at -20º C for 2 minutes, and 2M HCl was injected into the media through the rubber seal to release media-bound CO_2. Chambers were incubated at 4º C for 1 hour. Muscle strips were then thrice washed and weighed, and specific activity of ^{14}C in NaOH and media aliquots were determined as described above.

Statistical Analysis

All data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC). In Experiment 1, steer was the experimental unit and in Experiment 2, fetus was the experimental unit. For both experiments, glucose uptake and oxidation were determined from the same muscle or cell isolates, but from different wells. Values for glucose uptake and oxidation were normalized to the mean of the basal group and are expressed as means ± standard error.

RESULTS

Experiment 1. Incubation of myoblasts with insulin increased ($P < 0.05$) glucose uptake compared to basal conditions (Figure 1). Incubation with β2 agonist alone also increased ($P < 0.05$) glucose uptake from basal conditions to a degree similar to insulin. However, glucose uptake in myoblasts incubated with insulin and β2 agonist together did not differ from myoblasts incubated with insulin alone. Glucose uptake in myoblasts incubated with β1 agonist alone did not differ from glucose uptake in myoblasts incubated under basal conditions. Moreover, glucose uptake in myoblasts incubated with insulin and β1 agonist together was less ($P < 0.05$) than in myoblasts incubated with insulin alone.

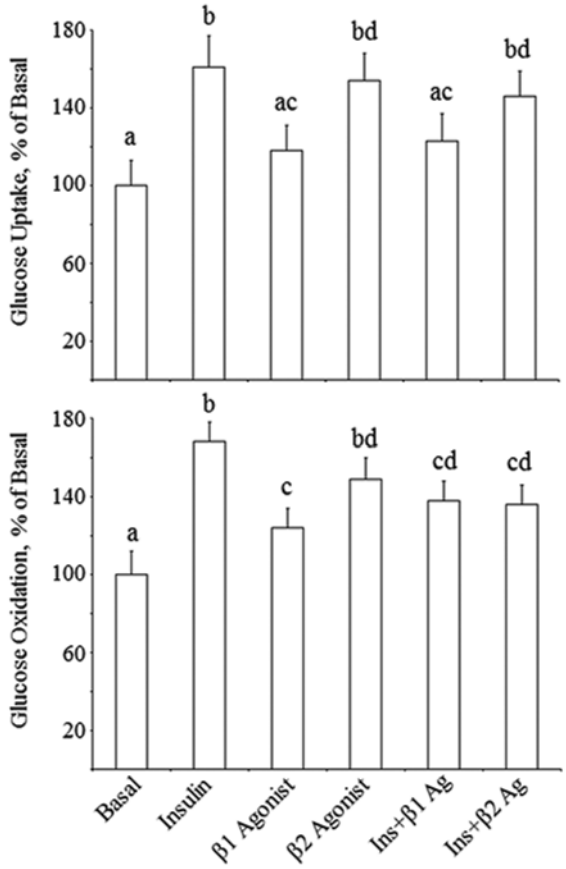

Figure 1. Glucose uptake and oxidation in primary bovine myoblasts during 20-min and 120-min incubations, respectively, spiked with one of the following treatments: No additive (basal), insulin (5 mU/ml Humulin-R), β1 agonist (1 μM Ractopamine HCl), β2 agonist (0.05 μM Zilpaterol HCl), β1 agonist + insulin, or β2 agonist + insulin. ^{a,b,c,d} means with differing supercripts differ ($P < 0.05$).

Incubation of myoblasts with insulin increased ($P < 0.05$) glucose oxidation compared to basal conditions (Figure 1). Incubation with β2 agonist alone similarly increased ($P < 0.05$) glucose oxidation compared to basal conditions. Conversely, glucose oxidation in myoblasts incubated with β1 agonist alone did not differ from myoblasts incubated under basal conditions. Furthermore, incubation of myoblasts with insulin and β1 agonist or with insulin and β2 agonist decreased ($P < 0.05$) glucose oxidation compared to myoblasts incubated with insulin alone.

Experiment 2. No interaction was observed between maternal treatment and incubation media. However, in-

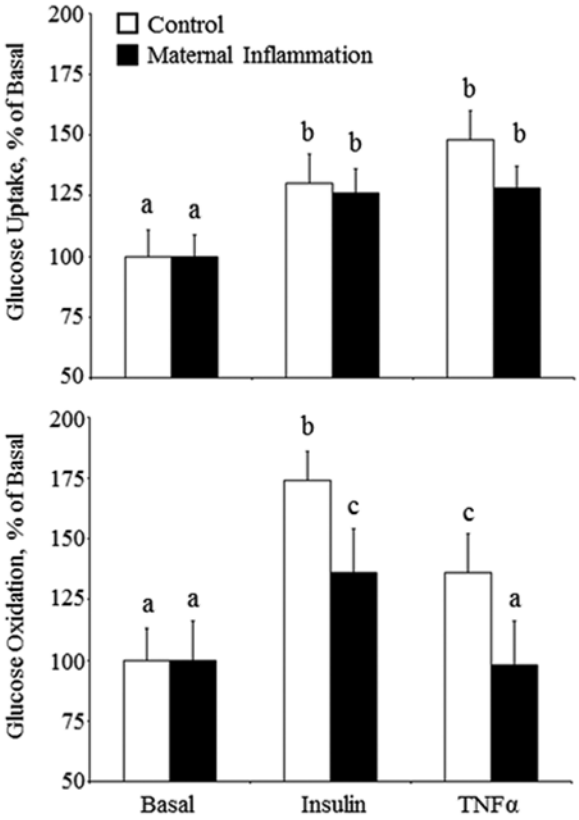

Figure 2. Glucose uptake and oxidation in primary fetal soleus muscle after maternal inflammation during 20-min and 120-min incubations, respectively, spiked with one the following treatments: No additive (basal), insulin (5 mU/ml Humulin-R), or TNFα (20 ng/ml hTNFα). [a,b,c] means with differing superscripts differ ($P < 0.05$).

cubation with insulin increased ($P < 0.05$) glucose uptake in fetal soleus muscle from control and LPS-treated ewes similarly (Figure 2). Moreover, glucose uptake rates in muscle incubated with TNFα was not different from muscle incubated with insulin in either treatment group.

Interacting effects between maternal treatment and incubation media were observed ($P < 0.05$) for glucose oxidation. Incubation in media spiked with insulin increased ($P < 0.05$) glucose oxidation in fetal muscle from both treatment groups, but the increase was greater ($P < 0.05$) in fetal muscle from control ewes than from LPS-treated ewes. Moreover, when muscle from control fetuses was incubated with TNFα, glucose oxidation was greater ($P < 0.05$) than when incubated under basal conditions but less ($P < 0.05$) than when incubated with insulin. Conversely, when fetal muscle from LPS-treated ewes was incubated with TNFα, glucose oxidation rates did not increase above basal conditions.

DISCUSSION

The findings from this study show that adrenergic and inflammatory mediators have a role in the metabolic regulation of developing muscle, as demonstrated by the effects on muscle stem cells (myoblasts) and fetal skeletal muscle fibers. Moreover, these effects can be insulin-dependent or insulin-independent. In primary bovine myoblasts, β_2 adrenergic agonists increased glucose uptake and oxidation in the absence of insulin, but β_1 adrenergic agonists had no effect on glucose uptake or oxidation, which was similar to findings in mature skeletal muscle (Cadaret et al., 2016). Unlike in mature muscle, however, insulin and β_2 adrenergic agonists together resulted in less glucose oxidation than either factor individually. In fetal soleus muscle, insulin-stimulated and TNFα-stimulated glucose oxidation was diminished by maternal inflammation earlier in gestation, although the capacity for insulin-stimulated or TNFα-stimulated glucose uptake did not appear to be affected. This shows that metabolic regulation by cytokines not only occurs in fetal muscle, but it can be diminished by fetal adaptations to chronic inflammation long after the inflammation has subsided.

β_2 adrenergic agonists appeared to stimulate more efficient glucose metabolism than β_1 adrenergic agonists, as the former increased both glucose uptake and oxidation rates in the primary bovine myoblasts. Since previous evidence shows that both stimulants increase growth efficiency (Centner et al., 2014), it is likely that β_1 and β_2 adrenergic agonists may be utilizing differing metabolic pathways. We speculate that β_1 adrenergic agonists are more efficient mediators of metabolism in adipocytes than myoblasts.

The combination of insulin and β_2 adrenergic agonists decreased glucose oxidation in primary bovine myoblasts, which suggests that β_2 adrenergic agonists work independently of insulin in myoblasts. Our previous study conducted in adult rat muscle showed that β_2 adrenergic agonists worked synergistically with insulin to enhance glucose metabolism (Cadaret et al., 2016), but this was not the case in our muscle stem cells. This indicates that prior to forming myofibers, myoblasts have a different mechanistic balance between metabolic regulators like insulin and the adrenergic system.

Stimulation of glucose metabolism by insulin was diminished in fetal muscle that had been exposed to chronic inflammation earlier in gestation. This indicates that chronic inflammation during development disrupts insulin signaling in skeletal muscle, and that the deficit remains even after the inflammation has been alleviated. This may help to explain previous findings that chronic inflammation during fetal development increases the risk of developing insulin resistance later in life (Hotamisligil et al., 1993).

This study also shows that incubation with TNFα after chronic inflammation decreases glucose oxidation when compared to controls. Our previous studies

show that acute exposure to inflammatory cytokines increases glucose oxidation in muscle even in the absence of insulin (Cadaret et al., 2016). However, the present study shows that chronic exposure to inflammation decreases this metabolic response and that subsequent exposure to inflammatory cytokines does not produce greater glucose oxidation. Thus, the benefit of this metabolic regulator is lost.

IMPLICATIONS

Our findings indicate that stress factors help regulate glucose metabolism in developing skeletal muscle and that their effects can be diminished by chronic over-exposure, even after exposure is alleviated. Furthermore, although ractopamine HCl and zilpaterol HCl are FDA approved for use as feed additives in animals, this study demonstrates that zilpaterol HCl is more effective in increasing metabolic efficiency.

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Effects of prescribed-burn timing on vigor of the noxious weed sericea lespedeza (Lespedeza cuneata) on native tallgrass range in the Kansas Flint Hills[1]

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ABSTRACT: We evaluated effects of annual, prescribed burning on vigor of the noxious weed, sericea lespedeza (*Lespedeza cuneata*; SL) in native tallgrass prairie over a 3-year period. We hypothesized that prescribed burning conducted during the growing season would selectively pressure SL, whereas locally-conventional, dormant-season prescribed burning would have no effect on SL. We used a 50-ha native tallgrass pasture infested with SL (initial basal frequency = 2 ± 1.3%, initial canopy frequency = 36 ± 3.4%) that was divided along watershed boundaries into 9 fire-management units (5 ± 2.6 ha) for this experiment. Burn units were assigned randomly to 1 of 3 prescribed-burning times (n = 3 / treatment): early spring (4/1; EARLY), mid-summer (8/1; MID), or late summer (9/1; LATE). Forage biomass, SL frequency, SL stem length, SL seed production, soil cover, and plant species composition were measured along single, permanent 100-m transects in each burn unit (100 × 30-cm^2 plot points/transect). Treatment and measurement date influenced forage biomass and SL stem length (treatment × time; $P \leq 0.01$). Forage biomass was not different ($P \geq 0.57$) between treatments on 7/19; however, forage biomass was greater ($P < 0.01$) in EARLY than MID and greater ($P < 0.01$) in MID than LATE on 10/10. Maximum stem length of SL was not different ($P \geq 0.78$) between treatments on 7/19. On 10/10, maximum SL stem length was less ($P < 0.01$) in MID and LATE than EARLY. Canopy frequency of SL was less (main effect, $P < 0.01$) in LATE than in EARLY and tended to be less in LATE ($P = 0.09$) than in MID. Whole-plant weight and seed production of SL at dormancy were greatly diminished ($P < 0.01$) in MID and LATE compared with EARLY. Frequency of bare soil, litter cover, and total basal plant cover were not different ($P \geq 0.38$) between treatments. Similarly, basal cover values of all grasses, major C4 tall grasses, all forbs, major wildflowers, and all shrubs were not different ($P \geq 0.24$) between treatments. We interpreted these data to indicate that prescribed burning during the growing season had strong suppressive effects on SL compared to locally conventional, early-season prescribed burning and produced no apparent detrimental effects on soil cover or non-target plant species. Post-fire regrowth was sufficient to prevent erosion and soil-moisture loss during the subsequent dormant season and would have allowed light grazing during the ensuing winter.

Key words: *Lespedeza cuneata*, prescribed fire, range improvement
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INTRODUCTION

Sericea lespedeza (**SL**) was introduced into the United States from Asia in the late 19th century as a soil-conservation measure. As a deeply-rooted perennial tolerant of poor soils, SL was a widely-used conservation plant in the US for nearly a century. Unfortunately, SL is highly invasive in the tallgrass prairie biome. Extremely high concentrations of condensed tannins cause beef cattle to avoid SL (Eckerle et al., 2011). A combination of canopy dominance, allelopathy, and prolific reproduction allows SL to rapidly propagate in native grasslands when seed is introduced. Seed can be transported great distances via the alimentary canal of wild and domestic herbivores and via farm equipment. In Kansas, SL has heavily degraded ~2,530 km^2 of pasture, primarily in the Flint Hills region (KDA, 2010). The result-

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ing damage to native habitats for wildlife and pasture quality for domestic herbivores has been devastating.

The predominant grazing management practice in the Kansas Flint Hills is known as *intensive-early stocking*. It involves annual spring burning in March or April followed by grazing with yearling beef cattle at a high stocking density for a relatively short period from April to August (Owensby et al., 2008). *Intensive-early stocking* involves removal of 40 to 60% of annual graminoid production; grazing lands then remain idle for the remainder of the year. Under this management practice, invasion by SL into the tallgrass prairie biome has steadily increased (Eddy et al., 2003). Vermeire et al. (2007) indicated that dormant-season spring fires likely stimulate SL germination by scarifying seeds lying on the surface of the soil. In contrast, Adams et al. (1982) reported plants that bloom and produce seed late in the growing season may respond negatively to prescribed burns conducted during the growing season. Cummings et al. (2007) reported also that application of growing-season prescribed fire at 3-yr intervals decreased the rate of SL invasion. Therefore, the objective of the study was to evaluate the effects of growing-season prescribed burning of native tallgrass range on vigor of sericea lespedeza.

MATERIALS AND METHODS

Procedures used in this study were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 3456).

A 50-ha native tallgrass pasture located in Geary Co., KS was used for our study. The site was historically grazed during the winter and spring by beef cattle; moreover, the infestation of sericea lespedeza on the site was problematic for the 20 yr period preceding our study (Tom Goudey, Geary Co. Noxious Weed Dept., Junction City, KS, personal communication).

The study site was divided along watershed boundaries into 9 fire-management units (5 ± 2.6 ha). Burn unit boundaries were delineated by mowing firebreaks (≈ 6 m wide) around each perimeter. Units were assigned randomly to 1 of 3 prescribed-burning times (n = 3 / treatment): early spring (4/1; **EARLY**) which served as a positive control; mid-summer (8/1; **MID**); or late summer (9/1; **LATE**).

Prescribed burns were carried out on or near target dates over 3 consecutive years when appropriate environmental conditions prevailed: surface wind speed = 8 to 20 km/h; surface wind direction = steady and away from urban areas; mixing height ≥ 550 m; transport wind speed = 13 to 33 km/h; relative humidity = 40 to 70%; ambient temperature = 13 to 30 °C; and Haines index ≤ 4. All prescribed burning activities were carried out with the permission of Geary Co. Emergency Services, Junction City, KS (permit no. 348).

Forage biomass, SL frequency, and SL maximum stem length were measured annually along single, permanent 100-m transects in each fire-management unit (100 × 30-cm^2 plot points/transect). Transects were laid out on a southwest-to-northeast gradient; transect ends were marked using steel fence posts. Transects were read at 1-m intervals on 07/19 and 10/10. A 100-m measuring tape was stretched from the southwestern end to the northeastern end of each transect. At 1-m intervals along each transect, biomass was measured according to Robel et al. (1970). In addition, a 30 × 30-cm plot was projected on the eastern side of transects at each point of measurement. Within the plot, presence of SL was noted (e.g., yes or no). If SL was present, stem height of the SL plant closest to the 1-m interval on the measuring tape was recorded. Stems were measured in cm from the surface of the soil to its maximum length by manually holding the SL stem erect.

A total of 100 mature SL plants were collected adjacent to permanent transects in each burn-management unit immediately after the first killing frost (average date = 11/2). Plants were clipped at ground level, placed into a labeled paper bag, and dried in a forced-air oven (96 h; 55º C). Individual plants in each sample (n = 100) were defoliated manually; seeds, leaves, chaff, and stems were placed collectively into a South Dakota Seed Blower (E.L. Erickson Products, Model B; 10-cm tube) to separate seeds. Cleaned seed was weighed to the nearest mg. Seed weight was converted to seed count assuming a density of 770 seeds/g (Vermeire et al., 2007; Vandevender, 2014). Average seed production was calculated by dividing the number of seeds by the number of SL plants in each sample (n = 100).

Plant species composition and soil cover were assessed along each permanent transect in mid-July using a modified step-point technique (Owensby, 1973). Transect points (n = 100) were evaluated for bare soil, litter cover, or basal plant area (% of total area). Plants were identified by species; basal cover of individual species was expressed as a percentage of total basal plant area.

Overall forage biomass, SL canopy frequency, and maximum SL stem length were analyzed as a completely random design (PROC MIXED, SAS Inst. Inc., Cary, NC). Class variables included fire-management unit, treatment, time of measurement, and year. The model included terms for treatment, time, year, and all 2- and 3-way interactions. Dry weight of SL plants, seed production, soil cover, and plant species composition were analyzed also as a completely random design with fire-management unit, treatment, and year as class variables. The model included effects for

treatment, year, and treatment × year. Least-squares means for the highest-order significant interactions were reported. When protected by a significant F-test ($P \leq 0.05$), means were separated using the method of Least Significant Difference.

RESULTS AND DISCUSSION

Total forage biomass and SL stem height were influenced by treatment and measurement date (treatment × time, $P < 0.01$; Table 1). Forage biomass was not different ($P < 0.01$) between treatments on 7/19 over the 3-year course of our study. In contrast, forage biomass was greater ($P < 0.01$) in EARLY than MID and greater ($P < 0.01$) in MID than LATE on 10/10. Application of prescribed fire treatments on 8/1 and 9/1 resulted in nearly complete removal of above-ground plant material; however, forage regrowth resulted in significant accumulations of biomass prior to seasonal plant dormancy. Near the end of the growing season (10/10), MID recovered to 38% of pre-fire biomass levels during the 10-wk period between treatment application and measurement. Similarly, LATE recovered to 22% of pre-fire levels over the 6-wk period between treatment application and measurement. We concluded that post-fire regrowth was likely sufficient to prevent erosion and soil-moisture loss during the subsequent dormant season and would have allowed light to moderate grazing during the ensuing fall and winter.

Maximum stem length of SL was not different ($P \geq 0.78$) between treatments on 7/19, at a time before growing-season prescribed burns were conducted (Table 1). In contrast, maximum stem length of SL in MID and LATE was less ($P < 0.01$) than half that in EARLY on 10/10. Growing-season prescribed fires had the effect of top-killing SL. Subsequent regrowth from the plant crown had limited opportunity to become reproductively mature prior to seasonal plant dormancy.

Canopy frequency of SL was not influenced by time of measurement, year, or any interaction between treatment, time, and year; therefore, main effects of treatment were reported (Table 2). Canopy frequency of SL before initiation of our study was not different ($P \geq 0.62$) between burn management units and averaged 36 ± 3.4% (data not shown). After 3 years of treatment, SL canopy frequency in MID and LATE was lesser ($P < 0.01$) than that in EARLY. In a related study, Ogden (2016) reported that basal frequency of SL on our site was greatest on EARLY (14.9%) and least on MID (5.9%) and LATE (3.0%). We interpreted this information to indicate that growing-season prescribed fires were successful in selectively pressuring SL; furthermore, it is probable that negative pressure on SL allowed native plants to colonize soil and canopy space formerly occupied by SL.

TABLE 1. Effects of the timing of prescribed burning on overall forage biomass and canopy frequency and stem height of sericea lespedeza (SL; *Lespedeza cuneata*) in native tallgrass rangeland

Evaluation date	Prescribed-burn timing	Forage biomass, kg DM/ha	SL maximum stem length, cm
07/19	Early spring (04/01)	4,971[a]	54.4[a]
	Mid-summer (08/01)	4,932[a, b]	54.5[a]
	Late summer (09/01)	4,738[a, b]	55.1[a]
10/10	Early spring (04/01)	4,138[b]	59.7[a]
	Mid-summer (08/01)	1,862[c]	23.3[b]
	Late summer (09/01)	1,040[d]	17.4[b]
	SE[1]	413.3	4.36
	P – treatment	< 0.01	< 0.01
	P – time	< 0.01	< 0.01
	P – treatment × time	< 0.01	< 0.01

[a, b, c, d] Means within a column with unlike superscripts are different ($P \leq 0.05$).

[1]Mixed-model SE associated with comparison of treatment × time means.

TABLE 2. Effects of the timing of prescribed burning on canopy frequency, whole-plant DM weight at dormancy, and seed production by sericea lespedeza (SL; *Lespedeza cuneata*) in native tallgrass rangeland

Item	Early spring burn (Apr 1)	Mid-summer burn (Aug 1)	Late-summer burn (Sep 1)	SE[1]	*P*-value[2]
Plant canopies containing, SL, % of total	49.9[a]	31.4[b]	20.3[b]	6.48	< 0.01
Whole-plant DM weight, mg/plant	3,954[a]	460[b]	163[b]	561.1	< 0.01
Total seed weight, mg/plant	924[a]	42[b]	1[b]	153.1	< 0.01
Seeds, no./plant	710.8[a]	32.6[b]	0.5[b]	117.82	< 0.01

[a,b]Means within a row with unlike superscripts are different ($P \leq 0.05$).

[1]Mixed-model SE associated with comparison of treatment main effect means.

[2]Treatment main effect.

Whole-plant DM weight of SL at dormancy, total seed weight per SL plant, and seed production per SL plant were greatly ($P < 0.01$) diminished in MID and LATE compared with EARLY (Table 2). Seed production in areas treated with mid-summer fire was less than 5% of that in areas treated with dormant-season spring fire. In areas treated with late-summer fire, seed production was less than 0.1% that of areas treated with dormant-season spring fire. Clearly, the capability of SL to reproduce via seed was sharply curtailed under a growing season fire regime.

TABLE 3. Effects of the timing of prescribed burning on graminoid basal cover, forb basal cover, occurrence of bare soil, and litter cover during mid-summer on native tallgrass rangeland

Item	Early spring burn (04/01)	Mid-summer burn (07/30)	Late-summer burn (09/01)	SE[1]	*P*-value[2]
Bare soil, % of total area	40.4	42.2	38.2	10.12	0.92
Litter cover, % of total area	49.3	47.9	49.9	10.09	0.98
Basal vegetation cover, % of total area	10.3	9.9	11.9	1.48	0.38
Total grass cover, % of total basal cover	84.4	86.2	88.0	2.71	0.44
Major C4 tall grasses,[3] % of total basal cover	49.3	47.9	49.9	10.09	0.98
Total forb cover, % of total basal cover	13.7	11.8	9.6	2.83	0.36
Major wildflowers,[4] % of total basal cover	0.48	0.93	1.11	0.377	0.24
Total shrub cover, % of total basal cover	1.87	1.94	2.44	0.605	0.60

[1]Mixed-model SE associated with comparison of treatment main effect means.

[2]Treatment main effect.

[3]Combined basal cover of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyium scoparium*), indiangrass (*Sorghastrum nutans*), and sideoats grama (*Bouteloua curtipendula*).

[4]Combined basal cover of catclaw sensitive briar (*Mimosa nuttallii*), dotted gayfeather (*Liatris punctata*), heath aster (*Symphyotrichum ericoides*), prairie coneflower (*Ratibida columnifera*), purple poppy mallow (*Callirhoe involucrate*), purple prairie clover (*Dalea purpurea*), roundhead prairie clover (*Dalea multiflora*), and white prairie clover (*Dalea candida*).

Frequency of bare soil, litter cover, and total basal plant cover were not different ($P \geq 0.38$) between EARLY, MID, and LATE (Table 3). Such values are generally indicative of healthy, normal tallgrass prairie ecosystems (Towne and Craine, 2014). Basal cover values of grasses, forbs, and shrubs were also not different ($P \geq 0.36$) between prescribed-burn treatments. In addition, functional species groups such as C4 tall grasses and major wildflowers were not influenced by the prescribed-fire regimes evaluated in our study. Collective basal cover of both groups was not different ($P \geq 0.24$) between treatments.

IMPLICATIONS

Compared to traditional, spring, dormant-season prescribed burning, prescribed burning during the summer months resulted in significant decreases in seed production by SL and suppressive effects on SL canopy frequency and stem length. In addition, growing-season prescribed burning did not result in measurable collateral damage to non-target plant species or soil cover. Growing-season prescribed burning may be an inexpensive and comprehensive means to control sericea lespedeza propagation. This manuscript presents the results from 3 yr of a 4-yr experiment.

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Effect of a low vitamin A diet on marbling and carcass characteristics of Angus cross and Simmental steers[1]

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ABSTRACT: Commercial Angus steers consisting of a minimum of 75% Angus genetics (Angus x Simmental) and purebred Simmental steers were used to evaluate the effect of a low vitamin A (VA) diet on growth and meat quality characteristics. After a 3 month backgrounding on a low vitamin A diet (1017 IU/kg), 64 steers (32 Angus cross, 32 Simmental) were allocated to one of two dietary treatments for finishing. The basal diet was low in VA (723 IU/kg). One treatment consisted of the basal diet with no supplemental VA (LVA), the other (CON) was supplemented with VA at the NRC recommended level of 2200 IU/kg DM. At the completion of finishing, steers were slaughtered at a commercial abattoir. Two strip loin steaks were collected from each steer to analyze meat quality characteristics including ether extract, color (L*, a*, b*), Warner-Bratzler sheer force, cook loss, subjective marbling score (scored by trained personnel), and pH. Camera image analysis from the slaughter plant was used for analysis of marbling, 12th s.c. fat, longissimus muscle area, hot carcass weight, kidney-pelvic-heart fat, and quality and yield grades. There was a difference ($P < 0.01$) in marbling score between the LVA Angus cross group and all other groups. The LVA treatment resulted in a 16% increase in marbling between treatments within the Angus cross group, but had no effect in Simmental cattle. Within the Angus cross cattle, the LVA treatment resulted in 26.6% of cattle grading higher (choice improving to prime) than their CON counterparts. Ether extract was highly correlated with marbling ($P < 0.01$), showing breed and treatment effects, but no interaction. Simmentals had greater ($P < 0.01$) REA and loin pH, whereas Angus cross steers had greater s.c. fat and KPH ($P < 0.01$) and subsequently greater yield grade. In conclusion, a LVA diet during finishing significantly increases marbling in Angus cross cattle. Angus cross cattle had greater s.c. fat and KPH, whereas Simmental cattle produce larger rib eyes. By removing VA supplementation from the finishing rations fed to Angus cross steers, the value of a carcass can be potentially increased, increasing profits for individual producers and the industry as a whole.

Key words: adipogenesis, Angus, ether extract, marbling, meat quality, Simmental, vitamin A
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INTRODUCTION

Marbling is one of the most influential factors that affect consumer preference in meat; generally palatability increases with increased marbling through factors such as tenderness, juiciness, and taste (Wang et al., 2009; Smith and Lunt, 2007). Marbling is also known as interfascicular or intramuscular adipose tissue, and is different than other types of fat because it is deposited alongside muscle fibers. Intramuscular fat deposition is more complex than that of subcutaneous fat in beef cattle; it is affected by the genetic propensity to marble, nutritional plane throughout life, and environmental factors. Vitamin A (VA) restriction has been shown previously to enhance marbling in cattle that genetically have a high propensity to marble such as Angus and Tijama cattle (Oka et al., 1998; Adachi et al.,1999), but in a different experiment using Limosin x Luxi (genetically lower propensity to marble) cattle, no difference was seen between VA treatments. (Wang et al., 2007) The ef-

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fect of VA on adipogenesis varies throughout the differing stages of adipose development. Retinoic acid (a VA derivative) affects adipogeneis by regulating the expression of adipogenic genes as well as decreasing lipid accumulation in adipocytes (Wang et al., 2016). In Angus cross steers VA restriction had no effect on average daily gain, dry matter intake, or feed efficiency (Bryant et al., 1999. In other studies there was a trend for VA restricted steers to exhibit lower ADG and feed efficiency, though this could be due to sub-clinical VA deficiency (Gorocica-Buenfil et al., 2007a,b, 2008). Restricting dietary Vitamin A may be a strategy to increase marbling without negatively impacting other production characteristics. The effect of VA restriction on cattle with high or low propensity to marble has yet to be compared, and may be an important aspect to consider when determining VA supplementation. The hypothesis of this study is that a low VA diet would increase marbling and improve meat quality characteristics while having no effect on production characteristics of Angus cross and Simmental steers.

TABLE 1. Ingredient and nutrient composition (DM basis) of TMR backgrounding and finishing diets fed

	Backgrounding, %	Finishing, %
Ingredient		
Brome Hay	15.00	0.00
Wheat Straw	30.00	10.00
Barley	10.00	0.00
Corn	20.00	60.00
CSB[1]	0.00	5.00
DDGS[2]	20.00	20.00
Supplement[3]	5.00	5.00
Nutrient Composition		
CP	15.00	14.68
NDF	48.24	25.51
ADF	26.02	10.33
Ca	0.69	1.01
P	0.32	0.53

[1]CSB = concentrated separator by-product (partially de-sugared beet molasses).

[2]DDGS = dried distillers grains with solutes.

[3]Supplement contained ground corn, limestone, urea, salt, monensin (Elanco, Greenfield, IN) vitamin D (3,000 IU/kg), and a trace mineral premix.

MATERIALS AND METHODS

Protocols described herein were approved by the North Dakota State University Animal Care and Use Committee. Steers were assessed for serum VA concentration at the beginning of backgrounding as well as the beginning and end of finishing to monitor VA status. Steers were weighed monthly and individual feed intake was recorded using the RIC feeding system (Insentec, Markness, the Netherlands).

Experimental Design and Treatments

The experiment had a 2 x 2 factorial treatment arrangement, comparing breed (Angus cross vs. Simmental) with VA treatment (LVA vs. CON). Simmental and Angus cross steers (n = 32 per breed) were obtained from the North Dakota State University beef unit. Previously, these steers had been grazing on summer pasture, therefor likely had high liver VA stores. The steers were fed a growing diet containing low concentrations (1017 IU/kg DM) of VA for 3 months (Table 1). This diet was designed to begin to deplete the liver VA stores, which can sustain serum retinol for 2 to 4 months. Steers were then moved to the North Dakota State University Beef Cattle Research Complex, where they were allocated to treatment. A total of 16 steers per breed were allocated to each nutritional treatment. The LVA treatment was the basal diet that contained 723 IU/kg of DM. The CON treatment was the basal diet plus a supplement containing the NRC recommended 2,200 IU of VA/kg of DM.

Slaughter and Meat Quality Analysis

Steers were slaughtered in 2 groups after 150 and 180 days of finishing, when they reached approximately 610 kg of BW. Steers were slaughtered at the Tyson slaughter facility (Tyson Fresh Meats., Dakota City, NE) where camera carcass data was collected by trained Tyson personnel. This data included kill date, grade date and time, lot number, carcass ID, hot carcass weight (HCW), sex, USDA Quality Grade (QG), USDA Yield Grade (YG), calculated yield grade (CYG), longissimus muscle area (LMA), marbling score, back fat depth, and kidney pelvic heart fat (KPH) percentage.

A boneless strip loin (IMPS 180) was collected from each carcass. The loin was transported back to the NDSU Meats Lab, where they were aged for 2 weeks. After aging, two 2.56cm steaks were cut from the center section of each loin. Steaks were analyzed for meat quality characteristics including subjective marbling score, marbling texture score, ether extract, Minolta color score, pH, drip loss, and Warner-Bratzler shear force. Subjective marbling score and marbling texture score was assigned by an experienced grader. Ether extract was performed by the NDSU Nutrition Lab following procedures described by the AOAC (2010). Minolta color score was scored with a Chroma Meter CR-410 (Konica Minolta, Tokyo, Japan). Meat pH was measured with a meat pH meter. (Hanna HI99163, Hanna Instruments, Woonsocket, RI) Steak

TABLE 2. Effects of dietary vitamin A concentration and breed on production characteristics

	Vitamin A[1]		Breed[2]			*P*-value[3]		
Item	LVA	CON	Simmental	Angus	SEM	VA	Breed	VA × Breed
BW, kg	610.45	597.41	584.0	623.4	12.2	0.4389	0.0236	0.5111
ADG, kg/d	1.52	1.44	1.45	1.51	0.027	0.0317	0.0827	0.2945
DMI, kg/d	11.10	10.58	10.29	11.39	0.25	0.1207	0.0016	0.2031
G:F	0.138	0.137	0.142	0.134	0.003	0.7499	0.0244	0.5896

[1]LVA = basal diet containing 1,017 IU/kg DM vitamin A; CON = basal diet supplemented at 2,200 IU/kg DM.

[2]Simmental = Pure bred Simmental steers; Angus = Commercial Angus steers containing > 75% Angus.

[3]VA = vitamin A level; Breed = Angus or Simmental.

weights were recorded, steaks were cooked to a final temperature of 71°C with a clamshell-style grill (George Forman Lean Mean Fat-reducing Grilling Machine™, Spectrum Brands Inc., Madison, WI), and cooked weights were recorded to calculate drip-loss. After cooking, steaks were placed on a metal tray to allow to cool to room temperature. After cooling to room temperature, six 1.27 cm cores were removed from each steak parallel to the muscle fiber orientation. These cores were sheared perpendicular to the muscle fibers using a Mecmesin BFG500N force gauge. (Mecmesin, Slinfold, West Sussex, UK)

Statistical Analysis

Data were analyzed using the mixed procedure of SAS (v. 9.4; SAS Inst., Cary, NC). Data were modelled in a 2x2 factorial treatment arrangement, with breed, treatment, and their interaction as fixed effects of interest; slaughter date was used as a fixed effect to remove variation. Steer served as the experimental unit. Means were separated using the LSMEANS procedure, and P-values ≤ 0.05 were considered significant.

RESULTS

Production characteristics

Average daily gain of steers in the LVA treatment was greater ($P = 0.03$) than the CON treatment, however, there was no effect of treatment ($P = 0.44$) on final body weight (Table 2). There was no effect of VA treatment on any other production measurement.

Final body weight was greater ($P < 0.0001$) for the Angus cross than Simmental steers (623.4 ± 11.44 and 584.0 ± 12.2 kg, respectively). Angus-cross steers exhibited greater DMI ($P < 0.01$) than Simmental steers (11.39 and 10.29 kg, respectively). Simmental steers were more efficient, having a greater ($P = 0.02$) gain to feed ratio than the Angus steers (0.1416 and 0.1338, respectively). Interestingly, there was no effect of breed or treatment on hot carcass weight ($P = 0.28$), as there was with final BW.

Meat Quality Characteristics

Angus-cross steers had greater ($P < 0.0001$) backfat and KPH fat than Simmental steers (Table 3). Conversely, Simmentals had a LMA ($P = 0.0002$). Subsequently, Angus cross exhibited greater Yield Grade ($P < 0.0001$), having a lower percentage of closely trimmed retail cuts. There was an interaction of breed and treatment on marbling score ($P = 0.008$). Within the Angus-cross steers, the LVA treatment had 16% greater marbling scores than CON Angus steers. There was no difference in marbling score between LVA and CON Simmental steers. This difference in marbling score was associated with Quality Grade differences. A greater proportion ($P = 0.02$) of Angus cross cattle graded choice than Simmental steers (Table 3). When examining prime carcasses, there was a significant interaction of breed and treatment, with more LVA Angus cross cattle grading prime than any other group ($P = 0.02$). The ether extract analysis is in agreement with marbling score, with observed main effects of treatment and breed ($P = 0.0401$ and $P < 0.0001$, respectively) but no interaction.

Cook loss was higher in the LVA treatment than the CON treatment (20.4 and 17.5%, respectively; $P = 0.0374$). Interestingly, there was a difference in meat pH between the Angus cross group and Simmental group ($P < 0.0001$). Finally, there was a tendency for steaks from Simmental steers to be more red (a* value) than their Angus cross counterparts ($P = 0.053$).

DISCUSSION

In contrast to prior studies, which have reported no difference or a reduction in growth with VA restriction (Gorocica-Buenfil et al., 2007a,b, 2008), we report an increase in ADG in steers fed a low VA diet. It is possible that restricted vitamin A affects the expression and

Table 3. Effects of dietary vitamin A concentration and breed on carcass characteristics of Simmental and Angus cross steers

	Vitamin A[1]		Breed[2]			*P*-value[3]		
Item	LVA	CON	Simmental	Angus	SEM	VA	Breed	VA × Breed
HCW, kg	385.00	387.70	381.60	391.10	20.700	0.7772	0.2838	0.7239
Sub-cutaneous fat, cm	1.18	1.13	0.93	1.38	0.055	0.8681	<.0001	0.7947
Marbling Score	578.96	517.38	469.32	626.93	40.200	0.0084	<.0001	0.0075
Ether Extract, %	6.32	5.14	4.28	7.18	0.409	0.0401	<.0001	0.2198
Rib Eye Area, cm^2	90.30	93.80	97.30	86.80	0.286	0.3013	0.0002	0.4233
Cook Loss, %	20.40	17.46	17.54	20.31	0.018	0.0374	0.064	0.7611
Shear Force, kg	2.26	2.48	2.46	2.28	0.110	0.1049	0.2729	0.7159
pH	5.46	5.47	5.49	5.43	0.011	0.3658	0.0001	0.1570
KPH, %	1.83	1.74	1.66	1.91	0.037	0.1084	<.0001	0.5064
USDA Yield Grade	2.87	2.71	2.26	3.32	0.106	0.4346	<.0001	0.6184
USDA Quality Grade[4]	1.95	2.11	2.29	1.77	0.164	0.1371	<.0001	0.2750
Select, %	17.20	20.00	37.9	0.00	0.064	0.8101	<.0001	0.8101
Choice, %	70.00	80.00	62.10	86.70	0.077	0.2963	0.0246	0.1505
Prime, %	13.80	0.00	0.00	13.30	0.083	0.0232	0.0233	0.0220
Minolta Color								
L*	43.10	43.10	42.90	43.30	0.681	0.8580	0.6201	0.3244
a*	25.50	26.00	26.20	25.30	0.315	0.1739	0.053	0.3188
b*	11.05	11.09	11.14	11.00	0.189	0.9448	0.5758	0.8939

[1]LVA = basal diet containing 1,017 IU/kg DM vitamin A; CON = basal diet supplemented at 2,200IU/kg DM.

[2]Simmental = Pure bred Simmental steers; Angus = Commercial Angus steers containing > 75% Angus.

[3]VA = vitamin A level; Breed = Angus or Simmental.

[4]1 = prime; 2 = choice; 3 = select.

production of hormones that effect hunger, and therefore intake. It has been shown in mice that supplementing VA downregulates leptin expression (Felipe et al., 2004). It is plausible that restricting VA intake upregulates leptin expression, therefore increasing intake and ADG.

The data from this study support the hypothesis that VA restriction during finishing increases marbling, however this was limited to cattle of high-marbling potential (i.e., Angus-cross steers). This is consistent with prior research on VA restriction in Angus-based cattle (Siebert et al., 2006; Gorocica-Buenfil et al., 2007a,b; Ward et al., 2012). This effect was not seen in Simmental cattle, which again is consistent with prior data showing that VA restriction does not affect marbling score in cattle with a low propensity for marbling (Wang et al., 2007). This experiment confirms that the impact of VA restriction on marbling is mitigated by breed propensity for intramuscular fat deposition. In this experiment, only Angus-cross steers on the LVA treatment produced prime-grade carcasses. It has been shown that retinoic acid plays large roles in both pre-adipocyte commitment and terminal maturation of adipocytes. This is accomplished by the regulation of many adipogenic nuclear receptors that are responsible for the regulation of adipogenic genes. Retinoic acid is a major ligand for many of these nuclear receptors, and is allocated differently throughout adipocyte development (Wang et al., 2016). Restricting VA may cause the reallocation of retinoic acid to different receptors that cause gene expression to be more favorable for adipose development. Ongoing research seeks to explain the mechanism by which VA restriction alters marbling.

Ether extract was highly correlated with marbling score, which is consistent with other studies (Dow et al., 2011). The greater cook loss observed in the LVA treatment steaks was likely due to their greater fat content (as measured by marbling score and ether extract), as fat has a lower melting temperature than the cooking temperature and can thereby liquefy and be lost during cooking.

IMPLICATIONS

Increasing marbling has the potential to add significant value to a beef carcass. According to the results of this research, feeding a low VA diet to cattle that have a genetic high propensity for marbling would function to increase the profits of cattle producers and packers without increasing cost of production. The increase in marbling without increasing other fat depots increases quality grade without a negative impact on yield grade. In cattle that have a lower propensity to marble, though there appears to be no effect on marbling, but VA restriction increased ADG. No adverse effects on

production characteristics or carcass characteristics were apparent when VA was not supplemented in the diet of either breed of cattle. Therefore, it may be beneficial to reduce or remove vitamin A supplementation from all cattle finishing diets, though care must be taken to avoid VA deficiency.

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Estimation of the requirement for water and ecosystem benefits of beef production on California rangelands[1]

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ABSTRACT: Among other agricultural sectors, beef production is accused of using large amounts of water, and in an effort to reduce water use, some recommend decreasing or halting meat consumption. Beef production water footprints vary and most do not consider the tradeoffs associated with ecosystem benefits provided by cattle on rangeland. A static model depicting water use for cow-calf production on California rangeland was developed on an Excel spreadsheet. Range water use for beef production was modeled at two UC Agriculture and Natural Resources (ANR) Research and Extension Centers: Hopland (HREC) and Sierra Foothill (SFREC), and at the USDA Forest Service San Joaquin Experimental Range (SJER). These three locations were chosen based on evapotranspiration (ET) zones and differences in forage production and rainfall. The model accounted for green water (i.e., water used for range forage production) and blue water (i.e., water used to grow alfalfa and irrigated pasture). As liters per kg of live weight, green water consumption was estimated to be 42,492 for HREC, 28,106 for SFREC, and 22,102 for SJER. Blue water consumption as liters per kg of live weight was estimated to be 4,631 for HREC, 12,784 for SFREC, and 9,140 for SJER. The model was sensitive to changes in range forage production and irrigated pasture use. Green water usage appears large; however, cattle consume less than 18% of the total water range forage plants use to grow. It is important to consider the water use associated with beef production in the context of ecosystem services cattle provide to rangelands, such as preventing grasslands from being converted to shrub lands, woodlands, or even forests, and the role grazing cattle play in managing and improving rangeland.

Key words: water, beef cattle, rangeland, ecosystem benefits
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INTRODUCTION

The prolonged drought in California raised concerns regarding the use of water for both animal and other agricultural sectors. Among agricultural sectors, beef production has particularly come under scrutiny. Accusations of large amounts of water use and land degradation by beef production lead to a common recommendation of reducing meat consumption in order to decrease water use (Marlow et al., 2009; Mekonnen and Hoekstra, 2010). However, such generalizations are based on estimates of the virtual water content of meat, which fails to describe the environmental relevance of water use in a product life cycle (Ridoutt et al., 2012). The virtual water content of a product refers to a volume of water that is consumed or polluted over the whole production chain; however, it does not consider what type of water (i.e., blue, green, grey) is used, or when and where it is used by the product (Hoekstra et al., 2011). The term water footprint refers to a multidimensional representation of the types of water consumed through a product's life cycle. Water footprints vary in the forms of water consumption represented, as well as other factors that affect the comparison of footprints for different products (Ridoutt and Pfister, 2010; Capper, 2011). Blue water is water consumed from surface and groundwater resources; green water is rainfall consumed through crop evaporation; and grey water is the volume of freshwater required for integrating water pollutants to a level accepted by water quality standards (Ridoutt and Pfister,

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2010; Capper, 2011; Hoekstra et al., 2011). The beef industry consumes different water types throughout the production chain and different production systems. In grazing-based beef production systems, green water will contribute a larger amount to the total water footprint than blue and grey water (Gerbens-Leenes et al., 2013). In beef production systems where animals spend more time on feed, blue water will contribute a larger portion of the water footprint due to the production of feed compared to the grazing system (Gerbens-Leenes et al., 2013).

To determine the water requirements of beef production in the U.S. and evaluate high water-use estimates, Beckett and Oltjen (1993) quantified developed water (i.e., blue water) used by all inputs of the beef production system. Water use was divided into drinking water, water for feed production (i.e., irrigation), and water for processing. Rainfall was not considered to be developed water; therefore, it was not included. The model Beckett and Oltjen (1993) developed depicted beef cattle production in the U.S. taking into account the variation in production systems across regions of the country.

The scope of this study differed from that of Beckett and Oltjen (1993) by focusing on cow-calf production on California rangeland rather than encompassing all stages of beef production within the U.S. This study did not include the feedlot or stocker segments of the beef production system as part of the water consumed by beef production. Instead, the blue and green water components of rangeland cow-calf production's water footprint were determined.

The objectives of this study were to revisit and update the analysis of Beckett and Oltjen (1993) to quantify water requirements for rangeland beef production in California at three different rangeland locations. An additional objective of this study was to highlight and compare ecosystem benefits associated with grazing cattle on rangeland, which was not previously mentioned by Beckett and Oltjen (1993). This study intended to also illustrate positive aspects of the rangeland beef production cycle and document the impacts on water use related to beef production on rangelands.

MATERIALS AND METHODS

A static model depicting water use for cow-calf production on California rangeland was developed on an Excel spreadsheet. Due to the variability in stocker cattle on California rangeland from year-to-year, the model focuses on cow-calf production. Personal communication with University of California Cooperative Extension (UCCE) Livestock Advisors confirmed the number of stocker cattle is variable with weather and is difficult to measure. The water accounted for included natural precipitation (i.e. green water), and water from surface or groundwater resources (i.e. blue water). Range water use for beef production was modeled at two UC Agriculture and Natural Resources (ANR) Research and Extension Centers: Hopland (HREC) and Sierra Foothill (SFREC), and also at the USDA Forest Service San Joaquin Experimental Range (SJER). These three locations were chosen based on their different evapotranspiration (ET) zones and differences in forage production and rainfall. The different components of ET encompass the processes in which water changes from liquid to gas form (Wilcox et. al, 2003). Evapotranspiration processes include evaporation from plant surfaces and soil, and transpiration from the plant (Wilcox et. al, 2003). Forage production and rainfall are routinely monitored throughout the growing season at these three locations. Water use for beef production was estimated for the ET zone in which each site was located.

TABLE 1. Dry matter intake (DMI) and assumptions of cattle on rangeland. Yearly DMI of breeding herd (i.e., cows, bulls, and replacement heifers) was calculated as 2% of body weight

Annual DMI for Breeding Herd			
	Location		
	SJER	HREC	SFREC
Breeding herd, animals	7,997	4,475	3,719
Total DMI, kg	30,650,546	17,149,096	14,255,615
Total DMI, kg/animal	3,833	3,833	3,833
kg alfalfa/animal	454	454	454
kg irrigated pasture/animal	227	0	454
kg rangeland/animal	3,152	3,379	2,926

For cow-calf production, our base assumptions were 85% calving, 18% replacement heifers per cow, and 5% bulls per breeding female. These assumptions were based on the assumptions of Beckett and Oltjen (1993). Average weight of cows, calves, replacement heifers and bulls in the model was 544, 163, 327 and 816 kg, respectively. Dry matter intake (DMI) of supplemental feed and forage provided by alfalfa and irrigated pasture was subtracted from the total DMI to determine rangeland forage intake (Table 1). No supplemental feed was considered in the analysis beyond alfalfa and irrigated pasture. Annual DMI of the breeding herd (i.e., cows, bulls, and replacement heifers) was calculated as 2% of body weight (BW). DMI as 2% of BW was consistent with the assumptions made by Beckett and Oltjen (1993). Increasing DMI to 2.1% (NRC, 2016) in the model may be appropriate and will increase the green water use by

approximately 6%. Breeding herd data, alfalfa and irrigated pasture DMI assumptions in each ET zone were based on personal communications with UCCE Livestock Advisors. Since the model is implemented on a spreadsheet, these parameters can be easily changed to evaluate parameter sensitivity.

Green Water

Green water considered in the model is assumed to be rainfall consumed through rangeland plant ET, then consumed by cattle as plants. At all three locations, average rainfall during the forage growing season exceeded average ET. Therefore, water use for rangeland forage production was assumed to be the average ET during the growing season. Rainfall and ET data were based on California Irrigation Management Information System (CIMIS) data for each location. Water used for rangeland forage growth that was consumed by cattle was estimated by dividing the herd rangeland DMI by the average rangeland dry matter production and multiplying by the total rangeland ET.

$$GW \quad \frac{Range\ DMI}{Range\ forage\ prod.} \times Range\ plant\ ET \quad (1)$$

where *GW* is total green water, *Range DMI* is the total DMI of range plants consumed by cattle, *Range forage prod.* is the total forage DM produced on rangeland, and *Range plant ET* is the total rainfall used for range plant growth. The model does not account for water that is potentially returned to the local environment from the animal in the form of urine and fecal water losses.

Blue Water

Blue water in the model is water consumed by animals as drinking water and water required to produce supplemental feed, specifically, irrigated pasture and alfalfa (Equation 2). Drinking water requirements of cattle were calculated according to the methods provided in Beckett and Oltjen (1993). Water used to produce alfalfa and irrigated pasture for beef production was modeled using data from the California Department of Food and Agriculture (CDFA, 2014) and the USDA, National Agricultural Statistics Service (2012). Harvested acres, irrigated acres, yield per acre, and applied water were used in the estimation of water use for the production of supplemental feed following the calculations used by Beckett and Oltjen (1993).

$$Blue \quad Drinking + Alfalfa + Pasture \quad (2)$$

TABLE 2. Water use by cow-calf operations on rangeland. L per kg estimates are based on kg of live weight produced on rangeland (i.e. cull cows and calves)

	Location		
	SJER	HREC	SFREC
Blue Water			
Drinking (m^3)	89,859.02	48,192.06	41,728.63
Alfalfa (m^3)	7,168,282.68	4,339,974.71	3,656,947.50
Irrigated Pasture (m^3)	8,219,602.35	0	6,371,947.99
Total Blue (m^3)	15,477,744.04	4,388,166.77	10,070,624.10
Total Blue (L/kg)	9,140.26	4,631.44	12,783.71
Green Water			
Total Green (L/kg)	22,101.78	42,492.11	28,106.44

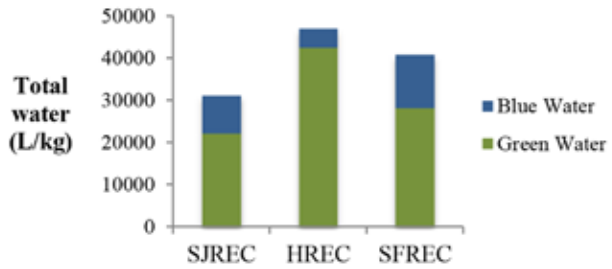

Figure 1. Blue and green water estimates (L of water/kg of live weight of beef production) on rangeland.

Where *Blue* is total blue water, *Drinking* is total drinking water consumed by the animals, *Alfalfa* is total water used to produce alfalfa fed to the cattle, and *Pasture* is total water used to produce irrigated pasture consumed by cattle.

RESULTS AND DISCUSSION

The estimates for the use of blue and green water for beef production on rangeland are provided in Table 2 and Figure 1. Blue water consumption as L per kg live weight was estimated to be 9,140 for SJER, 4,631 for HREC and 12,783 for SFREC. Green water consumption expressed as L per kg live weight was estimated to be 42,492 at HREC, 28,106 at SFREC, and 22,102 at SJER. Beckett and Oltjen (1993) found the number assumed for dressing percentage, boneless yield of beef carcasses, and water applied to and hectares of irrigated pasture to have the highest sensitivity to parameter changes in their model. From their study, Beckett and Oltjen (1993) concluded irrigated pasture was a leading major cost of water for beef production in the U.S. Similarly, in this study, irrigated pasture use is a sensitive parameter and factor influencing the blue water component of beef's water footprint. As ir-

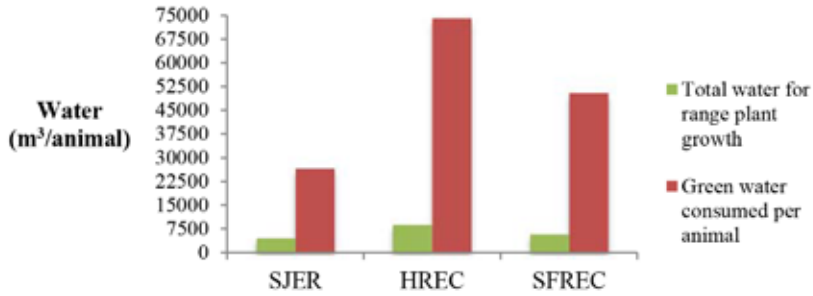

Figure 2. Of the total water used for range plant growth in the form of ET, cattle consumed 17.47, 12.14, and 11.83% at SJER, HREC, and SFREC, respectively.

rigated pasture consumption per animal increased, the total blue water per unit live weight increased.

Comparison of the model estimates to other studies must consider differences in production systems and system boundaries. Beckett and Oltjen (1993) estimated blue water as 3,682 L/kg of boneless beef. Capper (2011) reported blue water to be 1,763 L/kg HCW for US produced beef and did not include water used for processing. Both Capper (2011) and Beckett and Oltjen (1993) considered the entire beef production system to include the outputs of a finished animal. In this study, only the cow-calf sector of the industry was included, which considered the live weight of cull cows and calves to be the outputs of the system.

Green water use is sensitive to range production, and as forage production increased, the proportion of green water used by beef production decreased. Beef cattle production consumed less than 18% of the total water used by range plants through ET and less than 14% of the annual rainfall on rangelands (Figure 2). Of the water used by range plants, cattle are consuming a small portion of that water.

Previous studies that have expressed large water footprints for beef consumption have included green water estimates, which drive up numbers. For a US grazing beef production system, Gerbens-Leenes et al. (2013) reported a total water footprint of about 20,000 L/kg; however, approximately 18,000 L/kg of the total footprint is attributed to green water. Mekonnen and Hoekstra (2010) reported m^3/ton green water values as 19,102, blue water as 525, and grey water as 590, which is equivalent to 21,056 L/kg green water, 579 L/kg blue water, and 650 L/kg grey water for a US grazing beef production system.

The total water footprint for rangeland beef production appears large, but the highest source of that total is green water (Figure 1). Depending on the type of water and degree of stress on the water source, a large water footprint is not necessarily indicative of a large environmental impact (Ridoutt and Pfister, 2010; Ridoutt et al., 2012). Given that green water is sourced from rainfall and is not designated for another use, it is misleading to assign negative environmental impacts to rangeland beef production based solely on a large water footprint.

It is important to consider the water use associated with beef production in the context of the many ecosystem services livestock provide to our rangelands, such as preventing grasslands from being converted to shrub lands, woodlands, or even forests. Grazing cattle play an important role in managing and improving rangeland by benefiting individual plant and animal species and helping manage fire hazards. Ruminant livestock can graze areas that are not favorable for cultivation and growing crops, or land that is highly erodible (Oltjen and Beckett, 1996). If not used for grazing livestock, rangeland that is unable to be cropped may be utilized to support growing communities, housing developments, and road networks, or left unused and unmanaged with negative consequences. These changes can have multiple impacts on the hydrologic cycle including decreased infiltration, increased runoff, and increased water pollution (Donaldson, 2004).

The effects of grazing cattle and range management practices on water yield, water quality, slope stability, and erosion have been reported over fifty years of watershed research at HREC. The conversion of woodland to grassland significantly impacted the hydrology and sediment dynamics of watersheds, specifically decreasing runoff during storms (Burgy, 1968; Dahlgren et al., 2001). These changes resulted from an increase in grass cover (Murphy, 1976), retarded overland flow, and permitted more opportunities for infiltration (Burgy, 1968). However, grasses intercept far less rainwater than trees and shrubs, which can increase the amount of rainfall reaching the soil surface (Burgy and Pomeroy, 1958). Research from HREC suggests that agricultural production practices should maintain soil surface cover to enhance water infiltration and reduce surface runoff. Low ground cover leads to increased annual water yields, and increased summer and fall base flows (Dahlgren et al., 2001).

Additionally, maintaining grasslands and cattle grazing can be beneficial to vernal pool communities. Pyke and Marty (2005) presented data collected from a grazing enclosure study, which indicated that 3 years after the removal of grazing, un-grazed vernal pools dried an average of 50 days per year earlier than grazed control pools. Grazing plays an important role in maintaining the suitability of vernal pool hydrological conditions for fairy shrimp and California tiger salamander (*Ambystoma californiense*) reproduction (USFWS, 2004; Pyke and Marty, 2005). Additionally, livestock grazing can improve habitat for native annual forbs and grassland birds, control invasive weeds, and reduce fire hazard (UC ANR, 2015).

IMPLICATIONS

The water footprint estimated consisted of blue and green water. As a percentage of rainfall and range plant water use, the water used by rangeland beef production is small. Variables that impact the amount of water required by beef production include range production levels, and supplemented feed or irrigated pasture fed to animals, as seen previously by Beckett and Oltjen (1993). Comparing the water footprint of beef across studies must consider the production system boundaries and the types of water presented to avoid a misrepresentation of a large water footprint.

Rangeland beef production provides ecosystem benefits that should be considered in the water footprint. Rangeland research has provided information concerning the effects of cattle and range management practices on water yield, water quality, slope stability and erosion. Grazing cattle on rangeland is effective in reducing fire hazards, preventing the conversion of grasslands, and improving habits for wildlife species.

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Identifying hyperthermia in heat-stressed lambs and its effects on β agonist–stimulated glucose oxidation in muscle

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ABSTRACT: Heat stress is known to decrease value and production efficiency in food animals. Conversely, β agonists increase value due to increased muscle growth efficiency, but it is unknown how each of these factors impacts the other. In this study, we sought to determine how heat stress and β agonists affect glucose oxidation in muscle independently and in combination. Crossbred lambs were fed high-energy diets for 21 days containing one of three dietary β agonist treatments: no supplement, ractopamine HCl (β1 agonist), or zilpaterol HCl (β2 agonist). In addition, lambs were housed under one of two environmental conditions: thermoneutral (25°C, 15% RH) or heat stress (40°C, 35% RH). On the last day of treatment, two alternative temperature-measuring devices (infrared (IR) thermometer gun and IR camera) were compared to core body temperatures measured by rectal thermometer. Lambs were harvested on day 22 and intact soleus muscle strips were used to measure ex vivo glucose oxidation under basal and insulin-stimulated conditions. We found that ear and eye temperatures recorded with the IR camera and skin temperatures (sheared and unsheared) recorded with the IR thermometer guns (at higher emissivity) consistently correlated to core body temperatures measured with the rectal thermometer (r = ~0.6 to 0.7) and may represent non-invasive alternatives to rectal temperature for detecting hyperthermia in sheep. Surprisingly, we did not observe interactions among environmental treatment, dietary supplement, and incubation media for glucose oxidation rates. Exposure to heat stress for 21 days decreased ($P < 0.05$) skeletal muscle glucose oxidation by ~21%, dietary supplementation of β2 agonist for 21 days increased ($P < 0.05$) muscle glucose oxidation by ~15%, and addition of insulin to media during ex vivo incubation of muscle strips increased ($P < 0.05$) glucose oxidation by ~25%. Interestingly, dietary supplementation of β1 agonist had no discernable effect on muscle glucose oxidation. These findings show that heat stress reduces muscle glucose oxidation and β2 agonist increases it, although neither altered the impact of the other. Moreover, these effects were present 24 hours after treatments ended, which shows that heat stress and β agonist supplementation have lasting metabolic effects.

Key words: growth efficiency, metabolic regulation
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INTRODUCTION

Heat stress and β adrenergic agonists both elicit responses in tissues by activating adrenergic pathways. In livestock, heat stress is known to decrease growth and metabolic efficiency, and β agonist supplementation has been shown to improve growth performance and efficiency (Buntyn et al., 2016). However, little is known about how these two activators of the adrenergic system interact with each other. Catecholemines, such as epinephrine, are the natural ligand of the adrenergic system. These compounds interact with two classes of receptors, α adrenergic receptors and β adrenergic receptors (Mersmann, 1998). In recent years, supplementation of β-specific adrenergic agonists have benefited the livestock industry due to the increase in lean muscle mass and increase in total body weight that the supplements induce in feedlot animals (Elam et al., 2009; Montgomery et al., 2009). Two β agonist supplements are presently FDA-approved; the β1 agonist, ractopamine HCl, and the

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β2 agonist, zilpaterol HCl (Delmore et al., 2010; Boler et al., 2012). Boyd et al. (2015) hypothesized that the increase in muscle mass in zilpaterol-fed animals may lead to greater heat stress signals such as increased respiration and panting in cattle. However, after analyzing average and maximum body temperatures of these animals, they found that zilpaterol-fed animals actually maintained lower average body temperatures than control animals. These findings show that there is still much to be learned about how these compounds affect muscle metabolic function and growth. Additionally, it is important to understand how environmental stressors such as heat stress affect the efficacy of the supplements. Because of their contrasting individual effects, we hypothesized that heat stress and β agonist supplementation would have interacting influences on skeletal muscle glucose oxidation, a key determinant of metabolic efficiency. Our objective was to determine the impact that heat stress, β1 agonists, and β2 agonists have on muscle-specific glucose metabolism and how the effects of these factors interact. Furthermore, we sought to test the ability of alternative temperature-measuring devices to detect hyperthermia in heat stressed animals.

MATERIALS AND METHODS

Animals and experimental design

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Studies were performed at the UNL Animal Science Complex, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Columbia-Suffolk crossbred lambs averaging 11 months of age were purchased commercially. The study was performed in 2 blocks of 24 lambs each. After a 3-week acclimation period, all lambs were individually penned and fed identical high-energy base diets for 21 days and were housed under either thermoneutral (25°C, 15% RH) or heat stress (40°C, 35% RH) conditions. Additionally, each lamb received 1 of 3 dietary supplements: no supplement, ractopamine HCl (0.03996 g/hd/d), or zilpaterol HCl (0.025 g/hd/d) delivered in 200g ground corn added to the ration. Lambs were slaughtered on day 22.

Body temperature measurements

Ambient temperature and humidity in each pen were measured with a Hobo (Onset Computer Corporation, Bourne, MA) at the time of temperature measurements. Two identical rectal thermometers (ReliOn, Bentonville, AR) were used to measure core body temperature, and the readings were averaged.

Two infrared (IR) thermometer guns, designated gun A (Model FB61354, Fisher Scientific, Pittsburgh, PA) and gun B (Model TN418LD, Metris Instruments, Los Gatos, CA), and an IR camera (Model A655sc, FLIR Systems Inc., Wilsonville, OR) were used to measure outer body temperatures to compare to rectal temperatures. The guns were held ~2ft from the animal and 10-second average temperatures were recorded at three different locations: the center of nose between the nostrils, the sheared loin area of the back over the 14/15th ribs, and a non-sheared area (~3cm wool length) directly cranial to the sheared area. Temperature at each area was measured across a range of emissivity values, from 0.40-1.00. Images were captured with the IR camera at two different distances: 3-5 feet and 6-8 feet. Three forward-facing images were taken from each distance of each sheep. Images were analyzed using FLIR ResearchIR Max (FLIR Systems Inc.), and temperatures at the center of the nose between the nostrils, the center of the eye, and the center of the inside of the ear were averaged across the three images.

Soleus muscle isolation

Soleus muscles were collected tendon-to-tendon from the left hind limb at harvest and intact longitudinal strips were used to measure glucose oxidation. Muscle was washed in ice-cold phosphate buffered saline (PBS), dissected longitudinally, and strips were pre-incubated for 1 h at 37° C in gassed (95% O_2, 5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB) containing 0.1% bovine serum albumin (Gibco Life, Grand Island, NY). Media was spiked with either nil (basal) or 5 mU/ml insulin (Humulin-R; Ely Lilly), and 5mM glucose. Strips were then washed for 20 minutes in treatment-spiked KHB with no glucose.

Glucose oxidation

Rates for glucose oxidation were determined by oxidation of [^{14}C-U]-D-glucose as previously described (Cadaret, 2016) with some modifications. Muscle strips were placed in sealed dual-well chambers and incubated for 2 h at 37°C in treatment-spiked KHB with 5 mM [^{14}C-U]D-glucose (0.25 μCi/mmol). The adjacent well contained 2M NaOH to capture CO_2. Following incubation, chambers were cooled at -20°C for 2 min, 2M HCl was injected into the media through the rubber seal to release media-bound CO_2, and the chambers were incubated for 1 h at 4°C. Following incubation, muscle strips were weighed and NaOH was collected and mixed with UltimaGold scintillation

fluid to determine specific activity of $^{14}CO_2$ using liquid scintillation with a Beckman-Coulter 1900 TA LC counter (Brea, CA). Specific activity of the media was determined from three 10-µl aliquots mixed with 500µl distilled water and scintillation fluid. Radioactive compounds and scintillation fluids were purchased from Perkin-Elmer (Waltham, MA).

Statistical analysis

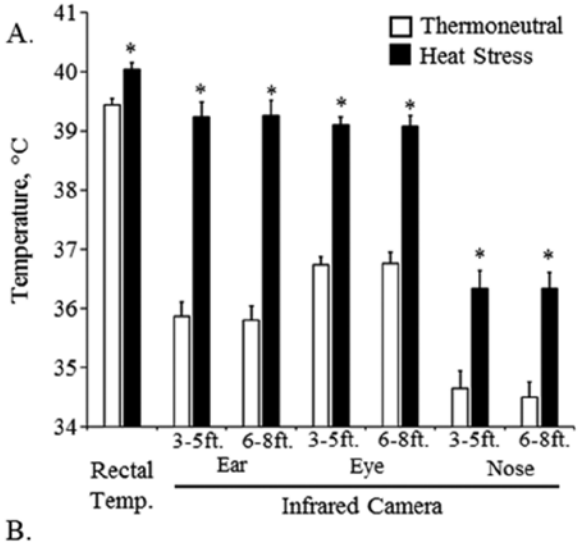
Temperature data were analyzed by one-way ANOVA for differences between thermoneutral and heat-stressed lambs using the GLM procedure of SAS (SAS Institute, Cary, NC). Pearson's correlation values between IR temperatures and rectal temperatures were also calculated using the CORR procedure of SAS. Glucose oxidation data were analyzed as a 2x3x2 factorial design by ANOVA using the GLM procedure of SAS, with environmental treatment (n = 24) and dietary supplement (n = 16) in the main plot and incubation media (n = 24) and all interactions in the subplot. For all outputs, lamb was the experimental unit. Data are presented as means ± standard error.

RESULTS

Hyperthermic measurements

Temperatures measured by rectal thermometer, IR thermometer guns, and IR camera were higher ($P < 0.05$) in heat stressed lambs than in thermoneutral lambs (Fig. 1). Typically, IR temperature measurements taken at the ear, eye, and back correlated well with rectal temperatures. However, IR temperatures measured on the nose of the animal were less correlative with rectal temperatures. Temperatures recorded by IR camera for each area did not differ due to distance from the animal, and correlations to rectal temperature were slightly greater for the ear than for the eye. For temperatures measured by IR thermometer gun, the greatest correlations were observed at higher emissivity levels (above 0.70), and correlations were not significant below 0.50 emissivity for either gun. Surprisingly, correlations to rectal temperatures were similar between sheared and unsheared areas of the back.

A.



B.

Area	Distance (ft)	Correlation w/ Rect. Temp (r)	*P*-val
Ear	3-5	0.678	<0.01
	6-8	0.660	<0.01
Eye	3-5	0.604	<0.01
	6-8	0.611	<0.01
Nose	3-5	0.381	0.06
	6-8	0.354	0.08

C.

Emissivity	Area	Correlation w/ Rectal Temp (r)	*P*-val
0.60	Sheared Back	0.600	<0.01
	Unsheared Back	0.649	<0.01
	Nose	-	NS
0.75	Sheared Back	0.715	<0.01
	Unsheared Back	0.707	<0.01
	Nose	0.517	<0.01
0.90	Sheared Back	0.705	<0.01
	Unsheared Back	0.700	<0.01
	Nose	0.575	<0.01

Figure 1. Body temperatures in control and heat-stressed lambs. A. Differences in temperature measured at the ear, eye, and nose of lambs by infrared camera. *Denotes differences ($P < 0.05$) between thermoneutral and heat stressed lambs for each area/distance. B. Pearson correlation coefficients between rectal temperature and temperatures measured by infrared camera. C. Pearson correlation coefficients between rectal temperature and temperatures measured by infrared thermometer.

Glucose oxidation

No interactions were observed between environmental treatment, dietary supplement, and incubation media, and thus only main effects are presented. As expected, incubation of muscle strips with insulin increased ($P < 0.05$) glucose oxidation rates in all muscle strips compared to incubation without insulin (Fig. 2). Exposure to heat stress for 21 days decreased ($P < 0.05$) glucose oxidation rates in muscle collected at slaughter the day after ending the environmental treatment. Dietary supplementation of ractopamine for 21 days did not affect muscle glucose oxidation rates, but dietary supplementation of zilpaterol for 21 days increased ($P < 0.05$) muscle glucose oxidation.

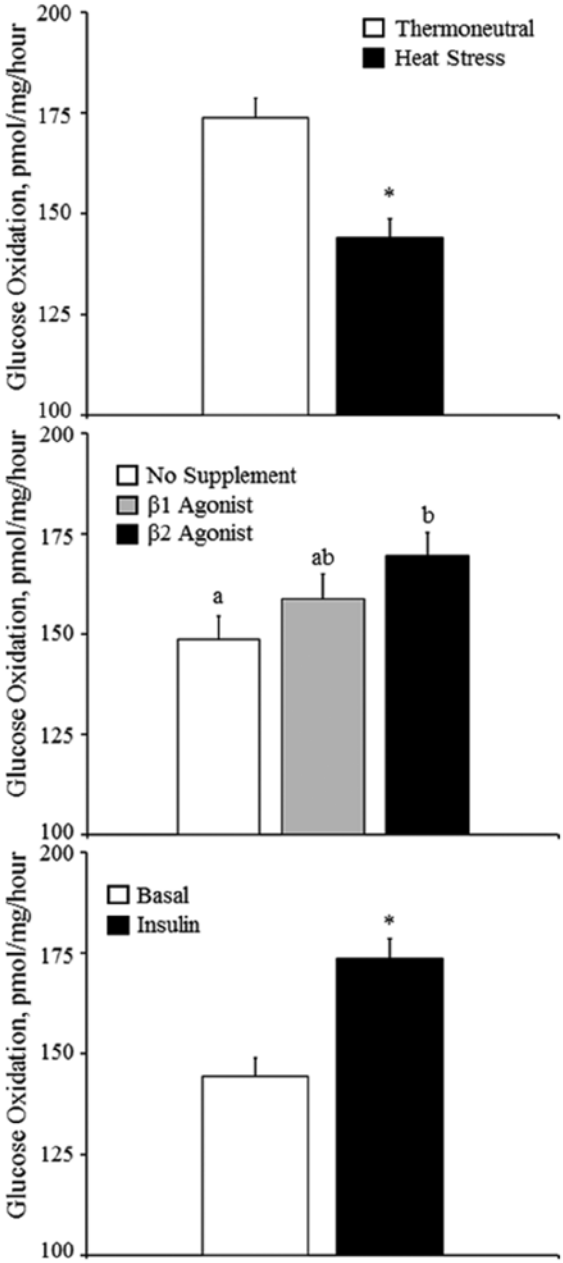


Figure 2. Glucose oxidation in primary soleus muscle from lambs housed under thermoneutral or heat stressed (40°C) conditions and fed a diet supplemented with β1 or β2 agonists. *Denotes differences ($P < 0.05$) between thermoneutral and heat stressed lambs in the top box and between basal and insulin-spiked media in the bottom box. [a,b]Denote differences ($P < 0.05$) among dietary supplements.

DISCUSSION

In this study, we show that hyperthermia can be detected in chronically heat-stressed livestock by infrared devices and that it is detrimental to metabolic efficiency. Surprisingly, chronic heat stress did not affect the metabolic benefit of β2 agonist supplementation, as muscle glucose oxidation was similarly decreased in heat stressed animals regardless of whether they received the dietary supplement or not. Likewise, β2 agonist supplementation improved muscle glucose oxidation in thermoneutral and heat stressed animals alike. Moreover, the respective effects of heat stress and β2 agonist supplementation on muscle glucose oxidation were observed under both basal and insulin-stimulated conditions. Together, these findings show that hyperthermic animals exhibit less metabolic efficiency, which helps to explain poorer growth performance under heat stress conditions. Moreover, β2 agonists are effective promoters of metabolic efficiency even in heat stressed animals, which contributes to their value as growth promoters.

Heat stress, like most physiological stressors, activates the adrenergic system, and thus it would be reasonable to postulate that animals experiencing chronic heat stress would be less responsive to β adrenergic supplements. However, we show that there was no interaction between heat stress and β agonist supplementation on skeletal muscle glucose metabolism. These surprising results indicate that the inhibitory and stimulatory effects of heat stress and β2 agonist supplementation, respectively, on muscle glucose oxidation occur through independent mechanisms. Although it is safe to assume that the β2 agonist is functioning solely through β adrenergic pathways, it is unclear whether the effects of heat stress are mediated by other components of the adrenergic system or by other regulatory systems altogether. It is important to note that the effects from heat stress and zilpaterol were observed 24 hours after ending both treatments. Although the possible effects of residual zilpaterol in the animal's system cannot be dismissed, it would appear from these findings that both factors have lasting effects on muscle glucose metabolism, which is not surprising when the adaptability of skeletal muscle is considered. Future studies may be able to show additional mechanisms by which heat stress and β2 agonist supplementation work to influence metabolic efficiency in muscle.

In order to evaluate the effects of hyperthermia on metabolic function in livestock, it is important to reliably identify hyperthermic animals. Core body temperature is traditionally estimated by measuring rectal temperature with a thermometer. However, safety concerns and animal disturbance may limit the use of this technique. In this study, we show that hyperthermia can be reliably detected in sheep by measuring body surface temperatures with infrared devices. In general, temperatures measured at the eye, ear, and loin area of the back by IR camera as well as the more affordable IR thermometer guns correlated well with rectal temperatures. Importantly, temperatures measured at the nose were highly inconsistent and did not correlate well with rectal temperatures, and thus should not be considered as appropriate measurements of body temperature. We speculate that the poor results from

the nose temperatures were due to the movement of air around the nose and differing amounts of moisture on the nose itself. Nonetheless, these IR devices are potentially useful tools to detect hyperthermia in livestock without entering the animal's pen or making physical contact. Moreover, additional research to normalize surface body temperature to core body temperature, these devices could be used as an alternative to rectal thermometers in clinical or production settings, which could improve safety and reduce animal stress.

IMPLICATIONS

In this study, we show that infrared devices can be used to detect hyperthermia in heat stressed animals and that hyperthermic animals exhibit reduced skeletal muscle glucose oxidation, which helps to explain performance deficits in hotter conditions. Moreover, β2 agonists improve muscle glucose oxidation but do not offset the heat stress-induced deficits, meaning that neither chronic heat stress or β2 agonist supplementation affects the animal's metabolic response to the other factor.

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Management of lethal recessive alleles while optimizing genetic gain in beef cattle[1,2]

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ABSTRACT: The beef cattle industry is a mainstay of U.S. agriculture with reproductive performance being the most important economic trait in a herd. Most beef cattle studies show fertilization rates around 90%, but calving rates to first service tend to be around 55%, suggesting some 35% of pregnancies are lost between fertilization and calving. Low frequency, recessive loss of function alleles at essential genes are thought to be associated with this embryo mortality. If producers knew which animals were carriers of recessive loss of function alleles, they could optimize mate selection to avoid carrier matings and thereby prevent the occurrence of homozygous affected calves. The objective of this study was to determine the optimum selection strategies given knowledge of loss of function alleles. The provided pedigree of an actual herd of 250 Angus cattle was used to simulate the impacts of different numbers and frequencies of loss of function alleles at essential gene loci using the mate allocation optimization software program MateSel. Three simulations were run modeling: 76 essential gene loci with loss of function alleles at a low frequency (Low 76), 7 essential gene loci with loss of function alleles at high frequencies (High 7), and 50 essential gene loci with loss of function alleles at random variations of high and low frequencies (Random 50). Within each simulation, different breeding strategies were explored: (1) selection against carrier animals as a class, and (2) selection against the occurrence of homozygous affected calves (i.e. carrier matings). The data indicate that while strong selection against carriers did result in significantly fewer homozygous affected calves, it came at considerable expense to genetic progress. However, selection against homozygous affected calves by mate allocation to avoid only the mating of loss of function carriers at the same essential gene locus also avoided homozygous affected calves but allowed for rates of genetic progress comparable to those obtained without consideration of loss of function alleles. Furthermore, a small number of essential gene loci can be relatively easily managed to minimize the loss of genetic gain irrespective of the loss of function allele frequencies. However, if loss of function alleles are present at a high number of essential gene loci, especially with a random distribution of loss of function allele frequencies, the power of a software program like MateSel is needed to optimally assign mate allocations to maximize genetic progress while minimizing the occurrence of homozygous affected calves.

Key words: lethal, loss of function alleles, mate selection, recessive
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INTRODUCTION

Management of inbreeding is an important focus for animal breeders because it can lead to reduced biological fitness of a population, also known as inbreeding depression. High levels of inbreeding resulted in the formation of British cattle breeds, such as Angus and Hereford, causing less heterozygosity or genetic diversity when compared to other breeds (Purfield et al., 2012) which can have a detrimental effect on performance traits (Carolino and Gama, 2008). Inbreeding has the largest negative impact on the total number of calvings through life, calf weight at 3 months of age, longevity, and number of calves produced up to 7 years (Carolino and Gama, 2008). As such, inbreeding has a significant impact on maternal-based traits in beef cattle. This is im-

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portant because reproduction plays an important role in a female's longevity within the herd. Fertilization rates in beef cattle are around 90%; however, average calving rate tends to be 55%, suggesting a 35% embryonic or fetal mortality rate (Diskin and Morris, 2008). If some portion of this loss is due to homozygous recessive loss of function alleles at essential genes, selection could be employed to avoid heterozygous carrier matings. MateSel (Kinghorn, 2011) is a software program that uses an evolutionary algorithm to make mating decision within a given population. Using this program, we tested three simulations. Each had a defined number of loci with various frequencies of loss of function alleles and employed two mating strategies: (1) selection against carrier animals as a class, and (2) selection against the occurrence of homozygous affected calves (i.e. carrier matings). We hypothesized that the second strategy of mate allocation to avoid carrier matings at the same essential gene locus, rather than indiscriminate selection against carrier animals as a class, would result in higher genetic gain while avoiding loses associated with affected calves, thus proving to be the more profitable strategy.

MATERIALS AND METHODS

Dataset Modeling

PopSNP (Kinghorn, unpublished data), a program which populates SNPs into a given pedigree dataset according to Mendelian segregation laws, was utilized to create three scenarios involving varying numbers of essential gene loci and loss of function frequencies. These were 76 loci with loss of function alleles at low frequencies (Low 76; mean 0.011, range 0.0004-0.069), 7 loci with loss of function alleles at high frequencies (High 7; mean 0.084, range 0.052-0.100), and 50 loci with loss of function alleles with random high and low frequencies (Random 50; mean 0.049, range 0.004-0.144) (Table 1).

Loss of function SNPs were populated into an Angus pedigree dataset with 85 male candidates, 169 female candidates, and 546 ancestors. A genome size of 30Mb and 29 chromosomes was modeled based on the size of bovine chromosomes. The Kosambi mapping function was utilized to calculate recombination fractions. A mutation rate of $2.2x10^{-9}$ was used to calculate generation and population size parameters (Liu et al., 2006). Any candidates or ancestors that would have received a homozygous recessive lethal genotype (aa) were assumed dead and not allowed within the population. The dataset included American Angus Association EPDs and economic selection index values for 21 traits for each candidate. To model a maternal index using existing values, we generated a combined maternal index ($M) by adding the $EN (dollar energy) index value to $B (dollar beef). The $B index emphasizes the postweaning performance and carcass value of an individual, while the $EN index emphasizes the energy cost of different mature cow sizes as well as lactation energy requirements.

TABLE 1. Allele frequencies for the three simulations with different numbers of loci

Number of Loci	Mean Frequency	Standard Deviation	Minimum Frequency	Maximum Frequency
7	0.0847	0.0151	0.0527	0.1001
50	0.0488	0.0307	0.0044	0.1436
76	0.0112	0.0125	0.0004	0.0695

Mate Selection

MateSel is a software program for tactical implementation of breeding programs, based on an evolutionary algorithm (Kinghorn, 2011). Initial changes were made to MateSel to manage lethal recessive alleles within a population, adding parameters LethalA and LethalG (Van Eenennaam and Kinghorn, 2014). LethalA is the predicted number of lethal recessive alleles in the progeny of selected matings across a chosen number of loci. Selecting against LethalA discriminates assigning matings to loss of function carriers (Aa) as a class, irrespective of which loci are heterozygous. The possibility of homozygous recessive parents at any essential locus within the population is zero, so the maximum value for LethalA is 0.5 + 0.5 = 1 per locus, if both parents are heterozygous. LethalA ranges in values from 0 to number of loci in a simulation. LethalG is the predicted number of lethal recessive genotypes (aa) within the progeny. Again, with the possibility of homozygous recessive parents being zero, the maximum value for LethalG is 0.5 * 0.5 = 0.25 per locus, if parents are heterozygous at all loci. LethalG ranges from 0 to 0.25 times the number of loci in the simulation (Van Eenennaam and Kinghorn, 2014). Selecting against LethalG is effectively selecting against the occurrence of lethal genotypes resulting from carrier (Aa) matings. This allows for the use of carrier sires provided they are not mated to females that are loss of function carriers at the same essential loci.

Simulation parameters

Mate selections were allocated to 125 matings with the highest resulting progeny index ($M) values. No sire could be mated more than 50 times. A target compromise between genetic gain (progeny index $M)

and inbreeding rate was set to 25 degrees in MateSel (see Kinghorn [2011] for explanation). Mate allocation runs were then performed with increasing weightings (0, 0.001, 0.01, 0.1, 1, 10, 100) to decrease the means of either LethalA or LethalG. Three scenarios were run: Low 76, High 7, and Random 50. A cost of $200 was assigned to the occurrence of a lethal "aa" genotype or embryonic mortality (Engelken, 2011). Profit per mating ($P) was calculated as $M – (LethalGx$200).

RESULTS

MateSel was used to provide mate allocations to the 125 females without awareness of loss of function alleles within the population, which provided a progeny index value ($M) of $82.38. This is referenced as the "zero" or no selection run.

High 7

In this simulation with 7 essential loci at high loss of function allele frequencies, the zero selection run resulted in a LethalG value of 0.036 (i.e. 3.6 lethal genotypes in 100 matings). Selection against all carrier parents to avoid LethalA in the progeny had little impact on genetic gain in this scenario, with a 6% decrease in the progeny index ($77.33) at the strongest weighting of 100 against LethalA when compared to the zero run. However, affected "aa" calves were still observed at 0.001, 0.01, 0.1, 1 weightings, with the 1 weighting giving the highest $P ($79.08). Selection against LethalG in the progeny had little impact on genetic gain, with 0% decrease at the 0.001 weighting, which also was sufficient to result in no homozygous affected calves (aa). $P was maximized ($82.38) at the lowest weighting (0.0001) against LethalA (Fig. 1a).

Low 76

In this simulation with 76 essential loci at low loss of function allele frequencies, the zero selection run resulted in a LethalG value of 0.0505. Selection against LethalA in the progeny had a detrimental impact on genetic gain, with a 13.9% decrease ($70.93) at the strongest weighting (100) when compared to the zero run. Again, affected "aa" calves were still observed at 0.001, 0.01, 0.1, 1 weightings. Selection against LethalG in the progeny achieved no homozygous lethal genotypes at the 1, 10, and 100 weightings at a ~ 2% decrease in genetic gain. Again, $P was maximized ($81.18) at the lowest weighting against LethalG (0.0001) and was always greater than weightings against LethalA (Fig. 1b).

Random 50

In this simulation with 50 essential loci at both high and low loss of function allele frequencies, the zero selection run resulted in the highest LethalG value of 0.155. Selection against LethalA in the progeny had the most detrimental impact on genetic gain, with a 22.8% decrease ($63.50) at the strongest weighting (100), while still resulting in some (0.002) homozygous lethal affected calves. Selection against LethalG achieved no homozygous lethal genotypes at the 10 and 100 weightings at an ~ 11.2% decrease in the rate of genetic gain. In this case, a weighting of 1 against LethalG maximized $P at $74.77, ~ $6.33 better than the $P resulting from a weighting of 1 against LethalA (Fig. 1c).

DISCUSSION

Within each simulation, the strategy of selection against homozygous recessive genotypes (aa) in the progeny allowed for greater genetic gain within the progeny index ($M) when compared to the strategy of selection against carrier parents (Aa). Increased genetic gain resulted from optimized mate allocation which matched genetically superior carrier individuals together provided they had loss of function alleles at different essential loci, maximizing the use of genetically superior carrier individuals. Furthermore, as weightings (0, 0.001, 0.01, 0.1, 1, 10, 100) to decrease the means of either LethalA or LethalG increased, the occurrence of homozygous recessive genotypes (aa) in the progeny decreased.

Selection to decrease LethalG was invariably more profitable than selection to decrease LethalA. This was particularly evident in the Random 50 simulation, with 50 essential loci at both high and low loss of function allele frequencies. Homozygous recessive genotypes (aa) were always produced, even at the strongest weighting (100) against LethalA. Selection against LethalG, however, was able to avoid affected calves entirely. In all scenarios, selection against LethalG was more profitable than selection against LethalA thus confirming our hypothesis, although the optimal weighting depended upon the number of loci and frequency of alleles.

With either a low number of essential loci (High 7) or low loss of function allele frequencies (Low 76), the mate allocations identified did not greatly decrease the rate of genetic gain ($M), nor the profit per mating. However, in the case of Random 50 with 50 loci and varied loss of function allele frequencies, a computer software program like MateSel would likely be required to optimally assign mate allocations to maximize profit.

As sequencing projects identify more essential genes and loss of function alleles, breed associations will need to develop policies on the management of

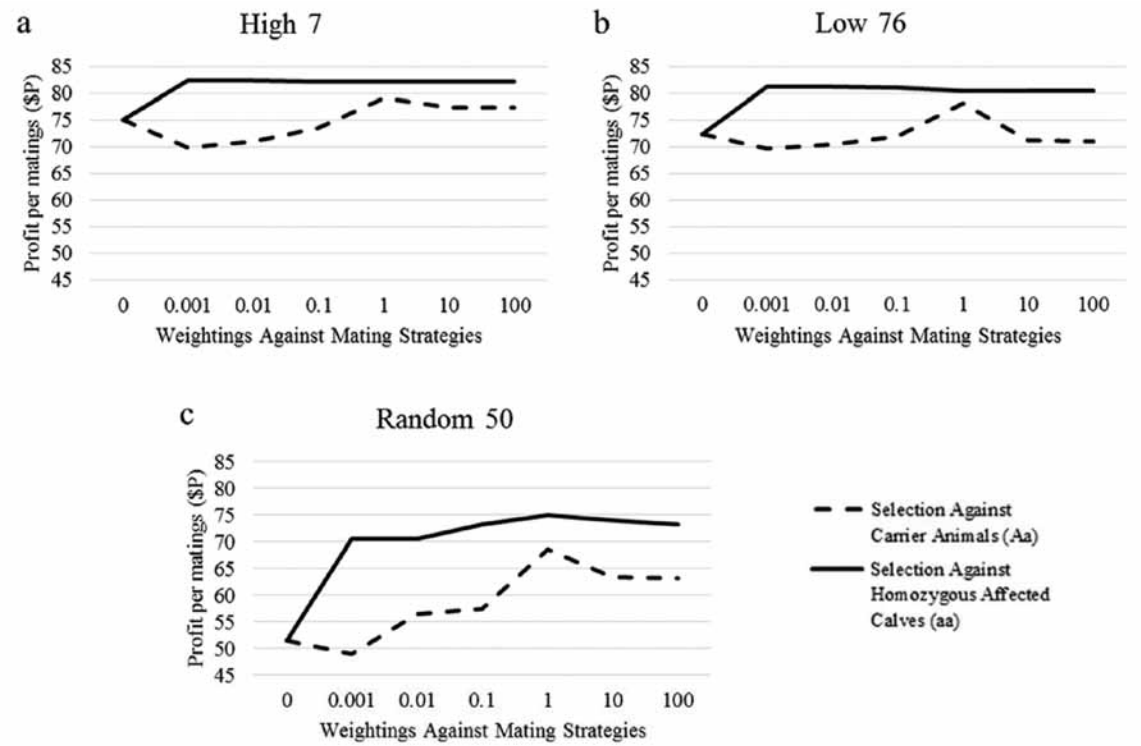

Figure 1. MateSel simulation results for two mating strategies, selection against carrier animals (Aa) and selection against homozygous affected calves (aa), using different numbers and frequencies of loss of function alleles at essential gene loci. a) High 7: Seven essential loci with high loss of function allele frequencies showing minimal decreases in profit per matings ($P) for selection against carrier animals (dashed line) compared to selection against homozygous affected calves (solid line) b) Low 76: Seventy-six essential loci with low loss of function allele frequencies showing slightly greater decreases in profit per matings ($P) compared to panel a for selection against carrier animals (dashed line) compared to selection against homozygous affected calves (solid line) c) Random 50: Fifty essential loci at both high and low loss of function allele frequencies showing significant decreases in profit per matings ($P) compared to panels a and b for selection against carrier animals (dashed line) compared to selection against homozygous affected calves (solid line).

lethal recessive alleles. In the long run, there may be some benefit in having breed association policies that put some selection emphasis against the occurrence of loss of function alleles. This would need to be balanced against the short term costs associated with forgoing the beneficial genetics associated with carrier animals.

IMPLICATIONS

MateSel can aid in mate allocation of cattle with loss of function alleles to optimize genetic progress while limiting the number of homozygous affected calves produced. Using a maternal index ($M) as the driver of genetic gain and genotypes of recessive loss of function alleles, MateSel can help producers avoid carrier matings and decrease embryo mortality. Mate allocation to avoid same-locus carrier matings is more profitable than arbitrary disqualification of all carriers as parents as has been the policy in some beef breed associations. The strategy of mate allocation to avoid carrier matings at the same essential gene locus is a more profitable short term approach to the management of loss of function alleles. As resequencing efforts expand, an increasing number of loss of function alleles and essential gene loci will be identified in beef cattle breeds. Computer software will be required to optimally manage loss of function alleles and genetic progress, thereby maximizing profit.

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Effects of late gestation supplementation, synchronization, and creep feeding in a spring calving beef herd in the Nebraska Sandhills

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ABSTRACT: A 3-yr study evaluated effects of late-gestation supplementation, postpartum progestin administration, and creep feeding on cow and calf productivity in a spring calving herd. In yr 1, 120 crossbred cows (479 ± 57 kg) were assigned to 1 of 4 late-gestation supplementation treatments, postpartum progestin or control, and 1 of 2 creep feed treatments in a 4×2×2 factorial arrangement of treatments in a completely random design. The four supplement (32% CP; 89% TDN) levels were 0 kg/(cow • d) Dec 1 to Mar 1, 0.41 kg DM/(cow • d) Dec 1 to Mar 1, 0.41 kg DM/(cow • d) Jan 15 to Mar 1, or 0.82 kg DM/(cow • d) Jan 15 to Mar 1. Administration of exogenous progesterone postpartum via a controlled internal drug release device (CIDR containing 1.38 g of progesterone) for 7 d and prostaglandin $F_{2\alpha}$ (5 mL Lutalyse) administered on d seven , or no CIDR. Unrestricted access by the calf to creep feed, which contained an intake limiter (Accuration) or no access to creep feed from July 15 to Nov 1. Steers were transported to a feedlot at the West Central Research and Extension Center near North Platte, Ne. Carcass data were collected 24 h following slaughter and final BW was calculated from HCW based on average dressing percentage of 63%. Carcass data included HCW, yield grade, LM area, marbling, and 12th rib fat. Higher levels of late-gestation supplementation increased cow BW ($P < 0.05$) and BCS ($P < 0.05$) precalving, but did not affect ($P > 0.12$) reproductive measures or calf performance. Exogenous progesterone administration postpartum did not affect ($P > 0.13$) cow or calf performance. Creep feed increased ($P < 0.01$) calf BW at weaning by 20 kg. Creep feeding calves increased ($P < .01$) carcass yield grade and 12th rib fat ($P < .01$) however, final BW and HCW were similar ($P > 0.10$).

Key words: beef cattle, creep feed, progesterone, supplementation
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INTRODUCTION

Extending the grazing season to include grazing dormant pasture decreases production costs (Adams et al., 1994). Research has determined supplemental RDP is necessary to maintain BCS of gestating cows grazing winter range in the Nebraska Sandhills (Stalker et al., 2007). Feeding supplement to cows grazing winter range during the last trimester of gestation has been shown to increase calf BW at weaning (Stalker et al., 2006, 2007) but it is not known if the timing of supplement feeding optimized progeny performance. Under-nutrition during gestation causes suboptimal conditions in the maternal uterine environment, which translate into depressed progeny performance (Wu et al., 2006). Cost savings may be achieved if supplement amount and duration supplement is fed were reduced. Further efficiency may be achieved if supplement is delivered directly to the calf and could potentially overcome detrimental effects of undernutrition during gestation. Supplementation directly to the calf significantly effects calf-weaning BW (Broadhead et al., 2016), but it is not known if this weight advantage will persist at slaughter. Administration of exogenous progesterone can shorten the postpartum interval (Lamb et al., 2008). If weaning occurs on the same d for all calves, those born to cows with a shorter postpartum interval will be older and therefore weigh more than contemporaries born to cows that become pregnant later in the breeding season.

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Objectives were to determine effects of late-gestation supplementation, postpartum progestin, and creep feeding on cow and calf productivity in a spring calving herd.

MATERIALS AND METHODS

All procedures and facilities were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (Project 921). A 3-yr experiment utilized 120 crossbred (¾ Red Angus, ¼ Simmental), March-calving cows (initial BW = 479 ± 57 kg) at the Gudmundsen Sandhills Laboratory, near Whitman, Nebraska. Cows were stratified by BW within age. Treatments were assigned randomly in a 4 x 2 x 2 factorial arrangement in a completely random design. The four supplement (32% CP; 89% TDN) treatments were 0 kg/(cow • d) Dec 1 to Mar 1 (**DM0**), 0.41 kg DM/(cow • d) Dec 1 to Mar 1 (**DM1**), 0.41 kg DM/(cow • d) Jan 15 to Mar 1 (**JM1**), or 0.82 kg DM/(cow • d) Jan 15 to Mar 1 (**JM2**). Administration of exogenous progesterone post-partum via a controlled internal drug release device (EAZI-Breed **CIDR** insert containing 1.38 g of progesterone; Zoetis Inc., Florham Park, NJ) for 7 d and prostaglandin $F_{2\alpha}$ (5 mL Lutalyse, Zoetis Inc.) administered on d seven (**CIDR**), or no progesterone (**NoCIDR**). Unrestricted access by the calf to creep feed which contained an intake limiter (Accuration, Purina Animal Nutrition LLC, Gray Summit, MO) from July 15 to Nov 1 (**Creep**) or no access to creep feed (**NoCreep**). The study began in December when cows were located to 1 of 8 upland range pastures (35 ha) where supplement treatments were delivered on a pasture basis 3 d/wk until March 1. Beginning March 1, cows were managed as a single group and fed hay until the end of the calving season. On May 28, CIDR inserts were administered to cows assigned to the CIDR treatment. On June 4, CIDR inserts were removed and cows were administered prostaglandin $F_{2\alpha}$. All cows were exposed to fertile bulls (1:25 bull:cow ratio) for 45 d, with breeding season ending July 15. The non-creep treatment occupied 1 pasture and creep treatments occupied 2 separate pastures. Creep treatment cattle were introduced into pastures containing creep feeders surrounded by panels with openings sufficient to admit calves but prevent cow entry (8 openings, 38 cm wide).

Cow BW and BCS were measured at the beginning and end of the supplementation period, prebreeding and at weaning. Calf BW was measured at birth, prebreeding, and weaning. Steer calves were slaughtered on June 14 (Tyson Fresh Meats, Lexington, NE.) Carcass data was collected 24 h following slaughter and final BW was calculated from HCW based on average dressing percentage of 63%. Carcass data included HCW, yield grade, LM area, marbling, and 12^{th} rib fat.

Cows were removed from the study for failure to wean a calf or become pregnant and were not replaced. Therefore, the number of cows decreased throughout the 3 yr study. Additional cows external to the experiment were introduced into pastures to maintain constant stocking rates during the experiment.

Cows assigned to the same winter supplement, CIDR and creep treatment within winter pasture served as the experimental unit. Replicated treatment means within yr were used for analyses of cow and calf response variables and carcass evaluation. Model fixed effects included winter supplement treatment, CIDR treatment, creep treatment, and all interactions. Year and residual error were included in the model as random effects. Data were analyzed with the GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC). Effects of treatment were considered significant when $P < 0.05$ as detected by Fischer's test. When the F-test was significant, least square means of treatments were separated using a t-test when $P < 0.05$. There were no interactions ($P > 0.18$) among treatments; therefore, data are reported as main effects.

RESULTS AND DISCUSSION

All supplemented groups (DM1, JM1, JM2) increased in BW from beginning of study to calving whereas DM0 decreased BW (P = 0.06; Table 1). Cows assigned to DM0 treatment had the greatest differences in BW after winter treatment to weaning. Even with this difference, they had similar BW at weaning as the beginning of winter treatment. This is most likely due to a compensatory gain. This result is in agreement with (Stalker et al., 2006) who reported cows receiving a protein supplement prepartum had greater BW and BCS precalving and similarly, nonsupplemented cows had greater BW and BCS gain during the postpartum period. The greatest loss in BW occurred between precalving (March) to start of breeding (May) for all 4 treatments. Other than calving BW, cows fed supplement maintained or increased in BW. Differences in BW among supplement treatments were most evident at the beginning of the breeding season where DM0 cows weighed the least ($P < 0.05$), JM1 and JM2 cows intermediate, with DM1 cows having the greatest BW. Cow BCS was lower ($P < 0.05$) at the start of the breeding season for cows not supplemented compared to DM1 and JM2 cows, with JM1 cows being intermediate. Despite decreased BCS over the winter treatment period for DM0 and loss in BCS for all groups

Table 1. Effects of winter supplement,[1] post-partum progesterone administration,[2] and calf access to creep feed[3] on cow and steer progeny productivity

	Supplement				Progesterone		Calf feed			P-Value		
	DM0	DM1	JM1	JM2	CIDR	No CIDR	Creep	No Creep	SE[4]	Supp	Progest	Feed
Cow BW, kg												
Initial (Dec)	479	494	483	479	481	487	482	485	9	0.35	0.37	0.63
Calving (Mar)	446[b]	507[a]	484[ab]	489[a]	482	481	476	487	12	0.06	0.95	0.03
Breeding (May)	434[b]	467[a]	449[ab]	455[ab]	449	453	448	454	9	0.04	0.49	0.34
Weaning (Nov)	480	500	489	487	486	492	492	486	10	0.37	0.41	0.42
Cow BCS[5]												
Initial (Dec)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0.1	0.88	0.76	0.81
Calving (Mar)	4.6[b]	5.0[a]	4.9[a]	5.1[a]	5.0	5.0	5.0	5.0	0.1	0.03	0.88	0.76
Breeding (May)	4.5[b]	4.8[a]	4.6[ab]	4.8[ab]	4.6	4.7	4.6	4.7	0.1	0.09	0.62	0.46
Weaning (Nov)	5.3	5.2	5.3	5.4	5.3	5.3	5.3	5.3	0.1	0.75	0.75	0.53
Calving date[6], d	83	86	84	83	83	86	86	83	3	0.79	0.10	0.13
Born in 21 d[7], %	81	74	85	84	82	80	76	86	7 0.06	0.45	0.65	0.04
Calving rate[8], %	98	98	99	98	99	97	96	100	3	0.96	0.33	0.08
Weaning rate[9], %	91	95	93	94	91	95	93	93	4	0.71	0.23	0.85
Pregnancy rate[10], %	79	93	93	85	88	87	90	85	7	0.23	0.88	0.11
Calf BW, kg												
Birth (Mar)	34	36	34	35	35	35	35	34	1	0.27	0.64	0.16
Breeding (May)	73	74	72	75	74	73	72	75	3	0.75	0.43	0.11
Weaning (Nov)	239	239	239	243	239	241	250	230	7	0.80	0.50	< 0.01
Live Weight	593	591	577	593	589	589	595	582	14	0.70	1.00	0.19
HCW, kg	373	372	365	373	372	370	375	366	8	0.73	0.79	0.17
12th rib fat, cm	1.37	1.30	1.37	1.40	1.32	1.40	0.59	1.50	0.03	0.84	0.50	< 0.01
Marbling[11]	439	454	453	448	432	465	458	439	19	0.79	0.03	0.24
LM, cm^2	90	90	90	84	90	90	84	90	1	0.75	0.98	0.29
USDA Yield Grade	2.9	2.9	3.0	3.2	3.0	3.1	3.2	2.8	0.20	0.51	0.52	< 0.01

[abc]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

[1]DM0: 0 kg/(cow • d) Dec 1 to Mar 1; DM1: 0.41 kg DM/(cow • d) Dec 1 to Mar 1; JM1: 0.41 kg DM/(cow • d) Jan 15 to Mar 1; JM2: 0.82 kg DM/(cow • d) Jan 15 to Mar 1 supplement (32% CP DM).

[2]CIDR: controlled internal drug release device (containing 1.38 g of progesterone; Zoetis Inc., Florham Park, NJ) for seven d and prostaglandin $F_{2\alpha}$ administered on d 7 from May 28 to June 4.

[3]Creep: unrestricted access by the calf to creep feed, which contained an intake limiter from July 15 to Nov 1.

[4]Standard error of the least squares mean (n = 4 observations per treatment replication [**3**/yr]).

[5]Scale of 1 (emaciated) to 9 (extremely obese).

[6]Day of yr calving occurred where January 1 = d 1.

[7]Cows calving within 21 d calculated by finding difference between birth date and breeding date and subtracting from 285.

[8]Calving rate calculated by dividing the number of cows to calve by the number of cows at the beginning of the production yr.

[9]Weaning rate calculated by dividing the number of cows to wean a calf by the number of cows at the beginning of the production yr.

[10]Pregnancy rate calculated by dividing the number of cows determined pregnant by the number of cows at the beginning of the production yr.

[11]Marbling: Small^{00} = 400, Small^{50} = 450, Modest^{00} = 500.

from calving to breeding, all groups had similar weaning BCS. Differences in BW and BCS caused by the supplementation treatment did not affect measures of reproductive efficiency such as calving date, calving rate, weaning rate, or pregnancy rate ($P > 0.20$; Table 1). Previous research evaluating effects of supplementing cows grazing winter range has demonstrated decreased weaning rate in cows not fed supplement (Stalker et al., 2006) but no effects in other studies (Stalker et al., 2007; Rolfe et al., 2011). Supplement treatments did not affect calf birth, breeding, or weaning BW ($P \leq 0.80$; Table 1). Previous research at the same location (Stalker et al., 2006, 2007; Rolfe et al., 2011) has consistently demonstrated decreased BW at weaning of calves born to cows not fed supplement grazing dormant winter range. Further research with a greater number of observations may be necessary to obtain definitive conclusions.

Progestin treatment did not affect ($P > 0.13$; Table 1) BW, BCS, reproductive measures, or calf BW. Reproductive measures may not have been affected due to the fact the herd already had acceptable repro-

ductive performance. Exogenous progesterone was not expected to affect cow BW or BCS. Potential increased calf age and therefore, increased weaning BW as a result of earlier conception in the breeding season due to progesterone administration was not realized ($P = 0.65$). Allowing calves access to creep feed increased ($P < 0.01$; Table 1) calf BW at weaning by 20 kg. Total amount of creep that disappeared from feeder was 1.2 kg DM/(calf • d). Late gestation supplementation to cows did not affect ($P > 0.51$; Table 1) calf carcass characteristics. Creep feeding calves did not affect ($P > 0.17$; Table 1) HCW, LM area, or marbling.. However, creep feeding increased ($P < 0.01$) yield grade and 12th rib fat ($P< 0.01$).

IMPLICATIONS

While feeding supplement during winter grazing increased cow BW and BCS, it did not affect reproduction or calf performance, thus increasing production costs without increasing returns. Using a CIDR in cowherds with existing acceptable reproductive performance may also increase costs without increasing returns. Feeding creep feed to calves is an effective means of increasing weaning BW, carcass yield grade and 12th rib fat but should be considered within the context of a cost/benefit analysis, which will be affected by market timing, as the benefit was not evident when steers were fed to slaughter weight.

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Impact of heifer development system and winter supplementation of May calving cows on subsequent growth and reproduction in two different breeding seasons

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Abstract: A 4-yr study was conducted to determine the impact of heifer development system on subsequent growth and reproductive performance in 2 breeding seasons, and to evaluate winter supplementation of May calving cows grazing dormant winter range or meadow on heifer progeny growth and reproductive performance. March-born (n = 225) and May-born (n = 258), crossbred (5/8 Red Angus, 3/8 Continental) heifers were stratified by BW and randomly assigned to 1 of 2 post-weaning nutritional treatments (2 pastures·treatment^{-1}·year^{-1}) from mid-January to mid-April. Heifers were offered ad libitum meadow hay (HAY) and 1.81 kg/d (32% CP, DM) supplement or allowed to graze meadow (MDW) and offered 0.45 kg/d supplement. In the March-born heifers, ADG during the treatment period was greater ($P < 0.01$) for HAY heifers than MDW heifers. Pregnancy rates were similar ($P = 0.92$) for HAY and MDW heifers. Furthermore, calving rate and the proportion of heifers that calved in the first 21 d was not different ($P \geq 0.33$) between treatments. Similar to the March-born heifers, May-born heifers on HAY treatment had greater ($P < 0.01$) ADG during the treatment period. Pregnancy rates were also similar ($P = 0.69$) for HAY and MDW heifers. Calving rate did not differ ($P = 0.88$) between treatments, although, the proportion of heifers that calved in the first 21 d was greater ($P = 0.02$) for MDW compared with HAY. Heifer development system did not impact pregnancy rate in the March or May replacement heifers; however, March heifer pregnancy rate was greater ($P < 0.01$) than May (87 vs. 70 ± 3%). The lower pregnancy rate in May heifers may be due to declining forage quality during the breeding season. To evaluate the effects of winter supplementation on heifer progeny, dams of May heifers grazed either dormant upland winter range with (RS) or without (RNS) a protein supplement or grazed dormant meadow with (MS) or without (MNS) a protein supplement. Birth BW tended to be lower ($P = 0.07$) in heifers born to RNS cows. However, calf BW at pre-breeding was similar ($P = 0.30$) among dam treatments. Birth to weaning ADG was greater ($P < 0.01$) in heifers born to MS cows than RNS cows. Furthermore, weaning BW was lower ($P < 0.01$) in heifers from RNS dams when compared with the other dam treatments.

Key words: calving date, fetal programming, heifer development
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INTRODUCTION

Traditional recommendations suggest heifers reach 55 to 65% of mature BW at the time of breeding. Due to the cost of retaining replacement heifers, more efforts have been made to devise economical heifer development methods. Previous studies have indicated heifers developed to lower target BW have comparable reproductive performance to heifers developed in higher input systems (Funston and Deutscher, 2004; Roberts et al., 2009; Funston and Larson, 2011). Furthermore, it has been reported heifers fed to 51 vs. 57% mature BW showed no difference in attaining puberty. However, heifers developed on corn residue had a reduced percentage that reached puberty compared with winter range or drylot (Martin et al., 2008). Post-weaning management systems can significantly affect progeny development as various rates of gain post-weaning

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have been shown to affect age at puberty and fertility in heifers (Patterson et al., 1992).

The amount of harvested and purchased feed required to sustain a cow herd in the Nebraska Sandhills can be reduced by a late spring calving date, in which the cow's nutritional demands better match forage quality and quantity. Protein is commonly supplemented to maintain cow BCS during winter grazing. Supplementing beef cows during late gestation can affect the lifetime productivity of the calf by altering post-weaning growth and heifer fertility (Martin et al., 2007; Larson et al., 2009).

Therefore, the objectives of the current study were to determine the impact of heifer development system on subsequent growth and reproductive performance in early and late summer breeding seasons, and to evaluate winter supplementation of May calving cows grazing dormant winter range or meadow on heifer progeny growth and reproductive performance.

MATERIALS AND METHODS

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

Heifer Management

A 4-yr study conducted at the Gudmundsen Sandhills Laboratory, Whitman, NE, utilized replacement heifers from 2 calving seasons. March-born (n = 225) and May-born (n = 258), crossbred (5/8 Red Angus, 3/8 Continental) heifers were stratified by BW and randomly assigned to 1 of 2 post-weaning nutritional treatments (2 pastures·treatment^{-1}·year^{-1}) from mid-January to mid-April. March heifers were weaned in October while May heifers were weaned in January. Heifers were offered ad libitum meadow hay (**HAY**) and 1.81 kg/d (32% CP, DM) supplement or allowed to graze meadow (**MDW**) and offered 0.45 kg/d of the same supplement. Prior to each breeding season, 2 blood samples were collected 10 d apart to determine pubertal status. Heifers with plasma progesterone concentrations greater than 1 ng/mL at either collection were considered pubertal. Heifers were synchronized with a single $PGF_{2\alpha}$ (Lutalyse, Zoetis, Florham Park, NJ) injection 5 d after being placed with bulls (1:20 bull to heifer ratio) for 45 d. Bulls were placed with March heifers May 23 and with May heifers on July 10. Pregnancy diagnosis was conducted via transrectal ultrasonography 40 d following bull removal.

Dam Management of May Cows

Dams of May heifers were utilized to evaluate the effects of late gestation supplementation on heifer progeny. May-calving cows grazed dormant upland winter range with or without supplement (**RS**, **RNS**, respectively) or dormant meadow with or without supplement (**MS**, **MNS**, respectively) from December 1 to March 29. Cows assigned to RS or MS over-winter treatment received the equivalent of 0.45 kg DM animal^{-1}·d^{-1} of a 32% CP supplement. Supplement was delivered 3 times/wk on a pasture (35.6 ha) basis. Following treatment, cows were managed as a single group and grazed native upland range the remainder of the year. Fertile bulls were placed with cows (1:20 bull to cow ratio) approximately August 1 for a 45 d breeding season. Five d after bull placement, cows were estrus synchronized with a single injection of $PGF_{2\alpha}$. Pregnancy was determined via rectal palpation or ultrasonography at weaning in early January.

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC), evaluating dam treatment and heifer treatment as main effects. Year and replication were considered random effects in heifer analysis while yr was the random effect in dam treatment analysis. Proportions of pubertal and pregnant heifers were analyzed using an odds ratio. Least squared means and SE of the proportion of pubertal and pregnant heifers by treatment were obtained using the ILINK function.

RESULTS AND DISCUSSION

March-born Gain and Reproductive Performance

Heifer BW, ADG, and reproductive performance are summarized in Table 1. Weaning and initial BW was similar ($P \geq 0.52$) between treatments. March-born HAY heifers had greater ($P < 0.01$) ADG during the treatment period than MDW heifers. However, spring (April 22 to May 22) and summer (May 22 to Sept. 10) ADG was greater ($P < 0.01$) for MDW heifers compared with HAY heifers. Post-treatment, prebreeding, and pregnancy diagnosis BW was greater ($P \leq 0.02$) for HAY heifers. Percent of mature BW prior to the breeding season was greater ($P < 0.01$) for HAY compared with MDW. However, pubertal status prior to breeding and pregnancy rate were similar between treatments ($P \geq 0.82$). Furthermore, calving rate and the proportion of heifers calving in the first 21 d was not different ($P \geq 0.33$) between treatments.

TABLE 1. Effect of over-winter treatment on March-born heifer gain and reproductive performance

Item	Heifer Treatment[1]		SEM	*P*-value
	HAY	MDW		
n	113	112		
Weaning BW, kg	201	200	6	0.52
Initial BW, kg	240	240	6	0.89
Post-treatment BW, kg	310	287	7	<0.01
Treatment ADG,[2] kg/d	0.78	0.51	0.03	<0.01
Spring ADG,[3] kg/d	0.21	0.55	0.19	<0.01
Pre-breeding BW,[4] kg	320	305	5	<0.01
Summer ADG,[5] kg/d	0.21	0.55	0.19	<0.01
Percent mature BW,[6] %	58	55	1	<0.01
Pregnancy diagnosis BW, kg	377	367	9	0.02
Pubertal,[7] %	64	69	19	0.82
Pregnancy rate, %	87	88	3	0.92
Calving rate,[8] %	85	83	3	0.61
Calved in 1st 21 d, %	79	74	4	0.33

[1]HAY = heifers received ad libitum hay and 1.81 kg/d supplement (32% CP DM) from Jan. 15 to April 15; MDW = heifers grazed meadow and received 0.45 kg/d supplement (32% CP DM) from Jan. 15 to April 15.

[2]Jan. 16 to April 22 (96 d) and includes the treatment period.

[3]April 22 to May 22 (30 d).

[4]May 22.

[5]May 22 to Sept 10 (111 d).

[6]Percent of mature BW at breeding based on mature cow size of 552 kg.

[7]Considered pubertal if blood serum progesterone concentration > 1 ng/mL.

[8]Percentage of heifers that calved.

March-born Calving Performance

Calf birth BW did not differ ($P = 0.70$) among treatments (30 vs 30 ± 1 kg; HAY vs MDW, respectively). The proportion of bull calves born was similar ($P = 0.32$) between HAY and MDW treatments. Additionally, calving ease, calf vigor, and dystocia rate were similar ($P > 0.62$) between treatments. Udder score, however, was more desirable ($P = 0.03$) for MDW vs. Hay heifers. Rebreed pregnancy rate was not different ($P > 0.52$) between HAY and MDW (86, 87 ± 8%; HAY, MDW) in addition to BW at rebreeding. Furthermore, calf BW at weaning was similar ($P = 0.35$) between treatments (205, 200 ± 4 kg; HAY, MDW).

May-born Gain and Reproductive Performance

Birth and weaning BW was similar ($P > 0.67$) between HAY and MDW heifers (Table 2). Additionally, ADG from birth to weaning did not differ ($P = 0.87$) between treatments. Initial treatment BW was also similar ($P = 0.87$) between treatments. Similar to March-born heifers, May-born heifers on HAY had greater ($P > 0.01$) ADG during the treatment period. Spring and summer ADG was greater ($P > 0.01$) for MDW heifers, likely due to a compensatory gain effect. Post-treatment, pre-breeding, and pregnancy diagnosis BW was greater ($P > 0.01$) for HAY compared with MDW heifers. Percent of mature BW prior to the breeding season was greater ($P = 0.01$) for HAY (58%) compared with MDW (54%). More May-born heifers on HAY were ($P = 0.02$) pubertal prior to breeding than MDW. Pregnancy and calving rates were similar ($P \geq 0.69$) between treatments, although, the proportion of heifers calving in the first 21 d was greater ($P = 0.02$) for MDW compared with HAY.

Heifer development system did not impact pregnancy rate in the March or May replacement heifers; however, March heifer pregnancy rate was greater ($P < 0.01$) than in May (87 vs. 70 ± 3%; Fig. 1). The lower pregnancy rate in May heifers may be due to declining forage quality during the later breeding season (Funston et al., 2016).

Heifer Progeny Performance of May Dams

Birth BW tended to be lower ($P = 0.07$) in heifers born to RNS cows (Table 2). However, pre-breeding BW did not differ ($P = 0.30$) among dam treatments. Average daily gain from birth to pre-breeding was greater ($P = 0.01$) in heifers from MS and RS cows when compared with RNS cows. Pre-breeding to weaning ADG was greater ($P = 0.03$) for MS than RNS but similar to MNS and RS heifers. Birth to weaning ADG was also greater ($P < 0.01$) in heifers born to MS cows than RNS cows. Weaning BW was lower ($P < 0.01$) in RNS heifers when compared with the other dam treatments. This is in agreement with Stalker et al. (2006) who also reported greater weaning BW in calves born to dams fed supplement prepartum. Initial treatment BW of heifer progeny involved in development study was lower ($P < 0.01$) in heifers from RNS cows when compared with the other dam treatments. Additionally, MS heifers tended ($P = 0.07$) to have a greater BW after the treatment period compared with RNS heifers. Heifer progeny ADG during the heifer treatment to summer period was not affected ($P > 0.46$) by previous dam treatment. At pre-breeding and pregnancy diagnosis, however, BW was similar ($P > 0.17$) among dam treatments. This is in contrast with data from Martin et al. (2007), which reported a greater pre-breeding and pregnancy diagnosis BW in heifers from protein-supplemented dams. Heifers born to MS cows had greater ($P < 0.01$) percent of mature BW than heifers from RNS cows. Pubertal status and pregnancy rate was similar ($P > 0.31$) across dam treatments. Furthermore, calving rate and the proportion of heifers calving in the first 21 d did not differ ($P > 0.36$) based on dam treatment.

TABLE 2. Effect of dam and heifer winter treatment on May-born heifer gain and reproductive performance

	Dam Treatment[1]						Treatment[2]			
Item	MS	MNS	RS	RNS	SEM	*P*-value	HAY	MDW	SEM	*P*-value
n	54	53	53	54			128	130		
Birth BW, kg	34[x]	34[x]	34[x]	33[y]	0.5	0.07	34	34	0.4	0.67
Pre-breeding BW, kg	94	95	96	91	3	0.30	94	94	2	0.99
ADG from birth to pre-breeding, kg	1.05[a]	1.02[a,b]	1.04[a]	0.98[b]	0.03	0.01	1.02	1.02	0.02	0.98
ADG from pre-breeding to weaning, kg	0.60[a]	0.57[a,b]	0.57[a,b]	0.56[b]	0.02	0.03	0.57	0.58	0.02	0.90
ADG from birth to weaning, kg	0.71[a]	0.69[a]	0.69[a]	0.67[b]	0.02	<0.01	0.69	0.69	0.02	0.87
Weaning BW, kg	194[a]	192[a]	192[a]	184[b]	4	<0.01	188	188	4	0.88
Initial Treatment BW, kg	193[a]	190[a]	190[a]	178[b]	6	<0.01	188	188	2	0.87
Post-Treatment BW, kg	251[x]	245[x,y]	245[x,y]	238[y]	7	0.07	261	232	7	<0.01
Treatment ADG,[3] kg/d	0.48	0.46	0.46	0.47	0.06	0.76	0.64	0.40	0.06	<0.01
Spring ADG,[4] kg/d	1.02	1.03	1.00	0.95	0.08	0.46	0.94	1.06	0.08	<0.01
Pre-breeding BW,[5] kg	350	345	341	338	11	0.27	353	335	10	<0.01
Summer ADG,[6] kg/d	0.52	0.55	0.52	0.51	0.12	0.73	0.48	0.57	0.11	<0.01
Percent Mature BW,[7] %	57[a]	56[a,b]	56[a,b]	54[b]	1	<0.01	58	54	1	<0.01
Pregnancy Diagnosis BW, kg	365	363	358	354	9	0.17	370	355	11	<0.01
Pubertal,[8] %	79	67	64	77	19	0.31	79	65	18	0.02
Pregnancy Rate, %	72	72	66	64	7	0.73	72	68	4	0.69
Calving Rate[9], %	67	65	64	62	7	0.96	67	65	5	0.88
Calved in 1st 21 d, %	68	63	80	75	8	0.36	64	79	6	0.02

[a,b,c]For Dam Treatment, means in a row with different superscripts are different ($P \leq 0.05$).

[x,y,z]For Dam Treatment, means in a row with different superscripts are different ($0.05 \leq P < 0.1$).

[1]MS = dams grazed dormant meadow and received 0.45 kg DM·animal^{-1}·d^{-1} 32% CP supplement; MNS = dams grazed meadow and received no supplementation; RS = dams grazed dormant range and received 0.45 kg DM·animal^{-1}·d^{-1} 32% CP supplement; RNS = dams grazed dormant range and received no supplementation.

[2]HAY = heifers received ad libitum hay and 1.81 kg/d supplement from Jan. 15 to April 15; MDW = heifers grazed meadow and received 0.45 kg/d supplement from Jan. 15 to April 15.

[3]Jan. 5 to May 10 (125 d), includes the treatment period.

[4]May 10 to July 9 (30 d).

[5]Determined July 9.

[6]July 9 to Sept 10 (63 d).

[7]Percent of mature BW at breeding based on mature cow size of 552 kg.

[8]Considered pubertal if blood serum progesterone concentration > 1 ng/mL.

[9]Percentage of heifers that calved.

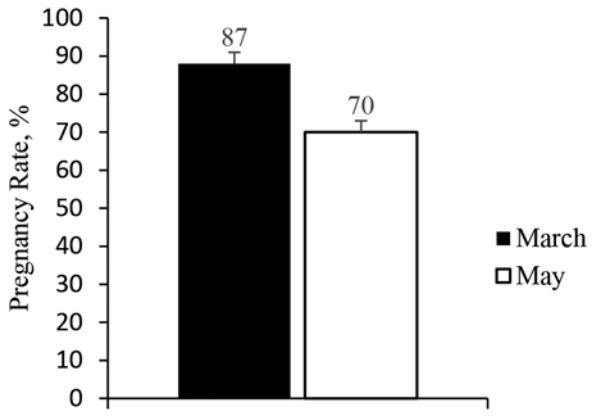

Figure 1. Comparison of March and May heifer pregnancy rates.

May-born Calving Performance

Calf birth BW was similar ($P > 0.32$) among development (29, 29 ± 1 kg; HAY, MDW) and dam (29, 28, 30, 29 ± 1 kg; MS, MNS, RS, RNS) treatments. The proportion of bull calves born did not differ ($P = 0.76$) across dam or development treatments. Additionally, calving ease, calf vigor, dystocia rate, and udder score were similar ($P > 0.12$) among treatments. Cow rebreed pregnancy rate was not different ($P > 0.19$) in either dam (94, 84, 60, 69 ± 10%; MS, MNS, RS, RNS) or development (83, 77 ± 8%; HAY, MDW) treatment. Cow BW at rebreeding was similar ($P > 0.31$) among treatments. Furthermore, calf weaning BW was similar ($P > 0.31$) between development treatments (168, 165 ± 5 kg; HAY, MDW) and among dam treatments (165, 159, 170, 165 ± 6 kg; MS, MNS, RS, RNS).

IMPLICATIONS

In the current study, heifer development system did not impact final pregnancy rates. Calf weaning BW and ADG from birth to weaning was less for calves from RNS cows compared with all other dam treatments. Therefore, a reduced input winter heifer development system is a viable option in both early and late summer breeding seasons. However, winter supplementation of May calving dams influences heifer progeny ADG from birth to weaning.

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Intensive late-season sheep grazing following early-season steer grazing is an effective biological control mechanism for sericea lespedeza (*Lespedeza cuneata*) in the Kansas Flint Hills[1]

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ABSTRACT: Mature ewes were used in a 4-yr study to evaluate effects of intensive late-season sheep grazing on vigor of sericea lespedeza (SL) in native tallgrass prairie. Pastures (n = 8; 31 ± 3.6 ha) infested with SL (initial basal frequency = 1.4%) were assigned randomly to 1 of 2 treatments: early-season beef steer grazing (1.1 ha/steer; initial BW = 258 ± 34 kg) from 4/15 to 7/15 followed by 60 d of rest (control; STR) or steer grazing from 4/15 to 7/15 followed by intensive grazing by mature ewes (0.2 ha/ewe; SHP) from 8/1 to 10/1. Ewes (initial BW = 65 ± 3.1 kg) were assigned randomly to graze 1 of 4 pastures; remaining pastures were not grazed from 8/1 to 10/1. Vegetation responses to treatment were measured along 4 permanent 100-m transects in each pasture. Herbivory of SL was monitored weekly in each pasture from 7/21 to 10/7. Herbivory of SL in SHP and STR after steer grazing was not different ($P = 0.51$). In contrast, SL herbivory following sheep grazing was greater ($P < 0.01$) in SHP than in STR. Herbivory of individual SL plants was greater ($P < 0.01$) in SHP than in STR by the end of wk 1 of the sheep-grazing period (10.6 vs. 0.5%); moreover, herbivory of SL steadily increased ($P \leq 0.01$) such that 92.1% of SL plants were grazed in SHP compared to 1.4% in STR by wk 8 of the sheep-grazing period. Whole-plant DM weight of SL at dormancy was less ($P < 0.01$) in SHP than in STR. Additionally, annual seed production by SL was less ($P < 0.01$) in SHP than in STR (114 vs. 864 seeds/plant). Pasture forage biomass was not different ($P = 0.76$) between SHP and STR after the steer-grazing period. Conversely, STR had more ($P < 0.01$) residual forage biomass than SHP at the end of the sheep-grazing period. Growth performance of beef steers grazing from 4/15 to 7/15 annually was not different ($P \geq 0.59$) between treatments. Our results were interpreted to suggest that intensive late-season grazing by sheep decreased vigor of SL. Late-season sheep grazing decreased forage biomass by 904 kg DM/ha compared with late-season rest; however, residual biomass was adequate to prevent soil-moisture loss and erosion during the dormant season. In addition, intensive grazing with sheep at the end of the growing season did not have detrimental effects on growth performance of steers grazing during the early portion of the growing season.

Key words: grazing, *Lespedeza cuneata*, sheep, tallgrass prairie
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INTRODUCTION

Sericea lespedeza (*Lespedeza cuneata*; **SL**) is a high-tannin, invasive, perennial forb in the Tallgrass Prairie ecosystem (Eckerle et al., 2010). Infestations of SL reduce native grass production by up to 92% through a combination of aggressive growth, canopy dominance, prolific seed production, and allelopathy (Kalburtji and Mosjidis, 1992; Dudley and Fick, 2003; Eddy et al., 2003). In Kansas, SL infests ~2,530 km^2 of pasture, primarily in the Flint Hills region (KDA, 2015). Herbicides retard the spread of SL but application is expensive and arduous, due to steep, rocky topography that is characteristic of the region (Eddy et al., 2003); moreover, herbicides are lethal to ecologically-important, non-target, native plant species.

Increased grazing pressure on SL by domestic herbivores may slow its spread and expedite some measure of biological control. Unfortunately, ma-

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ture plants contain high levels of condensed tannins, which reduce protein digestion by beef cattle and are a strong deterrent to grazing (Jones and Mangan, 1977; Eckerle et al. 2011a,b,c; Preedy et al., 2013). Conversely, small ruminants have greater tolerance for condensed tannins than beef cattle (Robbins et al., 1991; Hart, 2001; Pacheco et al., 2012). Sheep, in particular, appear less susceptible to certain plant toxins than beef cattle and may be useful to selectively pressure noxious weeds like SL (Ralphs et al., 1991; Henderson et al., 2012).

The most popular grazing management practice in the Flint Hills of Kansas involves annual spring burning followed by intensive grazing with yearling beef cattle from April to August (Owensby et al., 2008). Under this management practice, invasion by SL into the tallgrass prairie biome has steadily increased because SL flowers and produces seed from August to September (Cope and Burns, 1974; Koger et al., 2002; Eckerle et al. 2010). The absence of grazing pressure during this interval strongly promotes seed production, seed distribution, and continued invasion of the Flint Hills ecoregion by SL. This poses a serious threat to the pasture-based cattle industry in the Kansas Flint Hills (Wang et al., 2008). Therefore, the objective of our study was to evaluate the effects of late-season sheep grazing following locally-conventional steer grazing on vigor of SL, residual forage biomass, plant species composition, and subsequent performance of grazing steers.

MATERIALS AND METHODS

Animal care and handling practices used herein were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 3456). Our experiment was conducted during the 2013-2016 growing seasons at the Kansas State University Bressner Range Research Unit located in Woodson County, Kansas. Native tallgrass pastures (n = 8; 31 ± 3.6 ha) infested with sericea lespedeza (**SL**; initial basal frequency = 1.4%) were burned annually in April. Pastures were randomly assigned to 1 of 2 treatments: early-season grazing with beef steers (1.1 ha/steer; initial BW = 258 ± 34 kg) from 4/15 to 7/15 followed by rest for the remainder of the year (control; **STR**) or steer grazing from 4/15 to 7/15 followed by intensive grazing by mature ewes (0.2 ha/ewe; **SHP**) from 8/1 to 10/1. Ewes (n = 808 ± 6 annually; initial BW = 65 ± 1.7 kg) were assigned randomly to graze 1 of 4 assigned pastures; remaining pastures were not grazed from 8/1 to 10/1. Pasture treatment assignments were fixed for the 4-yr duration of the study.

Yearling beef steers were obtained from various commercial cattle growers in southeastern Kansas from 2012 to 2016. Steers were weighed individually before grazing began each April and were assigned randomly to pastures to create a stocking density of approximately 1.1 ha/steer. Steers were weighed individually again in late July.

Mature ewes were obtained from 2 commercial sheep operations located in western Kansas. Ewes were transported to the research site approximately 7/30 each year; they were weighed collectively by pasture groups before grazing began on 8/1 and after grazing was halted on 10/1. Final BW of sheep averaged 72 ± 3.1 kg. Sheep were monitored daily to assure they remained in assigned pastures and that fresh water was available continually. Death loss was < 2% annually and assumed to occur through predation or disease.

Vegetation responses to treatment were measured along 4 permanent 100-m transects (100 × 30-cm^2 plot points/transect) in each pasture. Transects were laid out on a north-south gradient; ends were marked using steel posts. Immediately before and immediately after sheep grazing, a 100-m measuring tape was stretched from the southern end to the northern end of each transect. At 1-m intervals along each transect, biomass was measured using a visual obstruction technique (Robel et al., 1970). A 30 × 30-cm plot was projected on eastern side of transects at each point of measurement. Within each plot, canopy type (i.e., grass- or forb-dominated) was noted, presence of SL was noted (e.g., yes or no), and evidence of herbivory was noted (i.e., obvious truncation of leaves or stems).

Weekly estimates of herbivory were conducted to evaluate grazing pressure on select forb species in each pasture. The species of interest were SL (*Lespedeza cuneata*), Baldwin's ironweed (*Vernonia baldwinii*), and ragweed species (*Ambrosia artemisiifolia, Ambrosia bidentata,* and *Ambrosia psilostachya*). Individuals of each species or group of species (n = 100/pasture weekly) were evaluated at temporary point transects. Point transect locations were determined randomly in control pastures. In treated pastures, point transects were located in areas where sheep grazing was observed to occur at the time of measurement. Evidence of herbivory (i.e., obvious truncation of leaves or stems) on individual plants was recorded.

Plant species composition and soil cover were assessed along 2 permanent transects in each pasture during October using a modified step-point technique (Owensby, 1973). Transect points (n = 100) were evaluated for bare soil, litter cover, or basal plant area (% of total area). Plants were identified by species; basal cover of individual species was expressed as a percentage of total basal plant area.

TABLE 1. Effects early-season grazing by beef steers followed by late-season grazing by sheep and time of measurement on pasture forage biomass, canopy-type frequency, and grazing activity in native tallgrass prairie infested with sericea lespedeza (*Lespedeza cuneata*)

	After steer grazing, before sheep grazing		After steer and sheep grazing		
Item	Steer grazing only[1]	Steer + sheep grazing[2]	Steer grazing only[1]	Steer + sheep grazing[2]	SE
Pasture forage biomass, kg DM/ha	2,467[b]	2,515[b]	3,156[a]	2,252[b]	158.2
Grass-dominated canopies, % of total canopies	85.6[a]	75.1[b]	78.9[a, b]	82.6[a]	3.71
Forb-dominated canopies, % of total canopies	14.4[b]	24.9[a]	21.1[a, b]	17.4[a, b]	3.71
Grazed grass canopies, % of grass-dominated canopies	74.4[b]	67.8[c]	8.8[d]	84.2[a]	3.07
Grazed forb canopies, % of forb-dominated canopies	21.2[b]	9.4[c]	9.5[c]	68.6[a]	5.18
Plant canopies with sericea lespedeza, % of total canopies	16.5[b]	32.8[a]	22.9[b]	32.2[a]	4.66
Grazed sericea lespedeza, % of plant canopies with sericea lespedeza	0.9[b]	3.0[b]	5.3[b]	89.6[a]	3.38

[a, b, c, d] Treatment × time – $P < 0.01$; within row, means with unlike superscripts are different ($P < 0.05$).

[1]Yearling steers grazed 4 pastures from 4/15 to 7/15 annually; pastures were rested for the remainder of the yr.

[2]Yearling steers grazed 4 pastures from 4/15 to 7/15 annually; mature ewes grazed these pastures from 8/1 to 10/1 annually.

A total of 100 mature SL plants were collected adjacent to permanent transects in each pasture immediately after the first killing frost (approximately 11/1 annually). Plants were placed into a labeled paper bag and partial DM was measured using a forced-air oven (96 h; 55º C). Individual plants in each sample were defoliated manually; seeds, chaff, and stems were placed into a South Dakota Seed Blower (E.L. Erickson Products, Model B; 10-cm tube) to separate seeds. Cleaned seed was weighed for each sample. Seed weight was converted to seed count assuming a density of 770 seeds/g (Vermeire et al., 2007; Vandevender, 2014). Average seed production was calculated by dividing the number of seeds by the number of SL plants in each sample (n = 100).

Line transect data were analyzed as a completely random design (PROC MIXED, SAS Inst. Inc., Cary, NC). Class variables included pasture, yr, time (i.e., pre-treatment or post-treatment), treatment, and transect. The model contained terms for treatment, time, and the 2-way interaction. Year was considered a random effect. Weekly herbivory indices were also analyzed as a completely random design (PROC MIXED, SAS Inst. Inc., Cary, NC). Class variables included treatment, pasture, yr, and wk. The model contained terms for treatment, wk, and the 2-way interaction; yr was considered a random effect.

Seed production by SL, DM weight of SL plants, soil cover, and plant species composition were analyzed as a completely random design, with treatment, pasture, and yr as class variables (PROC MIXED, SAS Inst. Inc., Cary, NC). Models included effects for treatment, yr, and treatment × yr.

Steer BW and ADG were analyzed as a completely random design (PROC MIXED, SAS Inst. Inc., Cary, NC). Class variables were treatment, pasture, and yr. The model included a term for treatment only and yr was considered a random effect. When protected by a significant F-test ($P \leq 0.05$), means were separated using the method of Least Significant Difference. Least-squares means for the highest-order, significant ($P \leq 0.05$) interaction term were reported.

RESULTS AND DISCUSSION

Pasture forage biomass was not different ($P = 0.76$) between treatments before the sheep-grazing period began (Table 1). In contrast, SHP had 904 kg less ($P < 0.01$) residual forage biomass than STR at the end of the sheep-grazing period (3,156 vs. 2,252 kg DM/ha for STR and SHP, respectively). Residual biomass in SHP was adequate to prevent soil moisture loss, allow prescribed burning the following spring, and to allow limited dormant season grazing if needed.

After the steer grazing period ended and before the sheep-grazing period began, the number of grass-dominated plant canopies was greater ($P < 0.01$) and the number of forb-dominated plant canopies less ($P < 0.01$) on STR than on SHP (Table 1). Conversely, proportions of grass- and forb-dominated canopies were not different ($P = 0.32$) between treatments at the end of the sheep-grazing period. The percentage of grass-dominated plant canopies that showed evidence of herbivory following steer grazing was relatively large and slightly different ($P = 0.03$) between STR and SHP; however, the percentage of grazed forb-dominated plant canopies following steer grazing was relatively small and slightly greater ($P < 0.01$) on STR than on SHP. At the end of the sheep-grazing period, STR had fewer ($P < 0.01$) grass- and forb-dominated plant canopies that showed evidence of herbivory than

TABLE 2. Effect of late-season grazing by sheep on herbivory of sericea lespedeza cuneata (*Lespedeza cuneata*; treatment × wk - $P < 0.01$, SE = 4.15)

Item	Steer grazing only[1]	Steer and sheep grazing[2]
Pre-Treatment,[3] % grazed	0.1[a]	0.6[a]
Week 1,[4] % grazed	0.5[a]	10.6[b]
Week 2,[4] % grazed	0.5[a]	22.4[c]
Week 3,[4] % grazed	0.9[a]	50.1[d]
Week 4,[4] % grazed	1.4[a]	64.8[e]
Week 5,[4] % grazed	2.5[a]	69.3[e]
Week 6,[4] % grazed	2.1[a]	78.4[f]
Week 7,[4] % grazed	3.5[a]	85.9[f,g]
Week 8,[4] % grazed	1.4[a]	92.1[g]

[a, b, c, d, e, f, g] Within row and column, means with unlike superscripts differ ($P < 0.05$).

[1]Yearling steers grazed 4 pastures from 4/15 to 7/15 annually; pastures were rested for the remainder of the yr.

[2]Yearling steers grazed 4 pastures from 4/15 to 7/15 annually; mature ewes grazed these pastures from 8/1 to 10/1 annually.

[3]Percentage of SL plants showing evidence of defoliation after yearling steers were removed and before sheep were allowed access to pastures.

[4]Percentage of sericea lespedeza plants showing evidence of defoliation each successive wk during a 60-d period in which mature ewes were grazed on 4 pastures.

SHP. We interpreted these data to indicate that steers strongly preferred to graze graminoid-dominated plant communities, whereas sheep did not appear to discriminate between plant canopy types.

Pastures assigned to SHP had greater ($P < 0.01$) SL canopy frequency than those assigned to STR after steer grazing and after sheep grazing (Table 1). Herbivory of SL was not different ($P = 0.51$) between STR and SHP following steer grazing and was generally minor. Conversely, herbivory of SL was much greater ($P < 0.01$) in SHP than in STR following sheep grazing. We interpreted these data to indicate that sheep displayed much greater preference for SL than steers. This conclusion was supported by weekly estimates of herbivory during the sheep-grazing period (Table 2). Herbivory of SL was not different ($P = 0.88$) and slight (<1%) in STR and SHP immediately following the steer-grazing period. In contrast, SL herbivory was greater ($P < 0.01$) in SHP than in STR by the end of wk 1 of the sheep-grazing period (10.6 vs. 0.5%); moreover, herbivory of SL steadily increased ($P < 0.01$) over time such that 92.1% of SL plants were grazed in SHP compared to 1.4% in STR by wk 8 of the measurement period.

Sheep also appeared to show preferences for other problematic forb species. Herbivory of Baldwin's ironweed and ragweed spp. was not different ($P \geq 0.69$) in STR and SHP immediately following the steer grazing period (data not shown). Conversely, herbivory of individual Baldwin's ironweed plants was greater ($P < 0.01$) in SHP than in STR by the end of wk 1 of the sheep-grazing period and was essentially complete by the end of wk 4. Sheep did not put a significant amount of grazing pressure on ragweed species until the end of wk 3 of the sheep-grazing period; thereafter, herbivory of ragweed species increased over time such that 57.6% of ragweed plants were grazed in SHP ($P < 0.01$) comparted to 0.6% in STR by the end of wk 8 of the sheep-grazing period.

TABLE 3. Effects of early-season grazing by beef steers followed by late-season grazing by sheep on whole-plant DM weight, total seed weight, and seed production of sericea lespedeza (*Lespedeza cuneata*), as measured immediately before seasonal plant dormancy

Item	Steer grazing only	Steer + sheep grazing	SE	*P*-value
Whole Plant DM Weight, mg/plant	4,424	1,443	357.6	< 0.01
Total seed weight, mg/plant	1,123	148	85.6	< 0.01
Seeds, no./plant	864	114	65.9	< 0.01

Tannin content of SL peaks during August and September (Eckerle et al., 2010; Preedy et al., 2013). This circumstance effectively protects the plant from herbivory prior to production of seed. Suppression of seed production may be a key to achieving control of SL. Whole-plant SL weight immediately after the first killing frost was 3-fold less ($P < 0.01$) in SHP than STR (Table 3). Annual seed production by SL and total seed weight were less ($P < 0.01$) in SHP than in STR (Table 3).

Occurrence of bare soil and litter cover were not different ($P \geq 0.63$) between treatments (Table 4). Conversely, total basal vegetation cover was greater ($P < 0.01$) in SHP compared to STR. Grass and sedge basal cover was greater ($P = 0.01$) and forb cover less ($P = 0.02$) in SHP than STR. Major C4 tall grasses decreased ($P < 0.01$) in SHP compared with STR; however, shrub and major wildflower basal cover were not different ($P \geq 0.20$)between treatments. Basal cover of SL was less ($P = 0.02$) in SHP than STR, whereas basal cover of Baldwin's ironweed tended to be less ($P = 0.09$) in SHP than STR. Basal cover of western ragweed was numerically less ($P = 0.18$) in SHP than STR. We interpreted these data to indicate that intensive, late-season grazing with sheep for the purposes of SL control was compatible with maintenance of healthy plant communities.

Initial BW, final BW, and ADG of beef steers grazing from 4/15 to 7/15 between 2012 and 2016 was not different ($P \geq 0.59$) between treatments (Data not shown). We concluded that SL control using late-

TABLE 4. Effects of early-season grazing by beef steers followed by late-season grazing by sheep on occurrence of bare soil, litter cover, and basal plant cover in native tallgrass prairie infested with sericea lespedeza (*Lespedeza cuneata*)

Item	Steer grazing only[1]	Steer + sheep grazing[2]	SE	*P*-value
Bare soil, % of total area	29.9	29.5	3.30	0.91
Litter cover, % of total area	60.6	58.9	3.49	0.63
Basal vegetation cover, % of total area	9.6	11.6	0.75	< 0.01
Grass and sedge cover, % of total basal cover	84.9	87.5	1.04	0.01
C4 grasses,[3] % of total basal cover	43.9	36.9	2.29	< 0.01
Forb cover, % of total basal cover	14.7	12.3	1.03	0.02
Major wildflowers,[4] % of total basal cover	1.06	0.75	0.236	0.20
Sericea lespedeza, % of total basal cover	3.62	2.12	0.633	0.02
Baldwin's ironweed, % of total basal cover	0.82	0.57	0.149	0.09
Western ragweed, % of total basal cover	4.47	3.80	0.502	0.18
Shrub cover, % of total basal cover	0.05	0.04	0.030	0.82

[1]Yearling steers grazed 4 pastures from 4/15 to 7/15 annually; pastures were rested for the remainder of the yr.

[2]Yearling steers grazed 4 pastures from 4/15 to 7/15 annually; mature ewes grazed these pastures from 8/1 to 10/1 annually.

[3]Combined basal cover of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyium scoparium*), indiangrass (*Sorghastrum nutans*), and sideoats grama (*Bouteloua curtipendula*).

[4]Combined basal cover of blue wild indigo (*Baptisia australis*), (catclaw sensitive briar (*Mimosa nuttallii*), heath aster (*Symphyotrichum ericoides*), plains wild indigo (Baptisia bracteata), prairie coneflower (*Ratibida columnifera*), purple poppy mallow (*Callirhoe involucrate*), purple prairie clover (*Dalea purpurea*), and white prairie clover (*Dalea candida*).

season intensive sheep grazing did not degrade subsequent performance by beef steers grazing during the early portion of the grazing season.

IMPLICATIONS

Late-season, intensive sheep grazing on native tallgrass prairie allowed comprehensive biological control of sericea lespedeza. In addition, late-season intensive grazing by sheep resulted in significant residual forage biomass, was compatible with conscientious ecosystem stewardship, and did not interfere with steer growth performance – a primary source of revenue for beef producers in the Kansas Flint Hills.

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Effects of early administration of Ralgro to Holstein calves on feedlot performance and carcass characteristics[1]

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ABSTRACT: Our hypothesis was that early administration of a growth implant on 1 d old Holstein calves will increase ADG and feed efficiency without negatively impacting health or carcass characteristics. At approximately 1 d of age, 1,248 male Holstein calves (initial BW 41.2 ± 0.2 kg) were utilized in a completely randomized block design with a 2 × 2 factorial arrangement of treatments and assigned to one of four treatments (n = 312 per treatment): 1) calves received an implant at the beginning of the hutch phase on d 0 and at the beginning of the wean phase on d 92 (IMP-IMP), 2) received an implant on d 0 only (IMP-NOIMP), 3) received an implant on d 92 only (NOIMP-IMP), or 4) did not receive any implants in either the hutch or wean phases (NOIMP-NOIMP). Calves were individually housed in hutches for 92 d, weaned, and on d 169 all calves were implanted and managed similarly until harvest. Calves were individually weighed on d 0, 92, 110, 169, 251, and at harvest. Administration of implant during the wean phase did not improve DMI, ADG, or G:F ($P \geq 0.13$). Calves implanted at the beginning of the hutch phase had greater ($P = 0.03$) DMI between d 169 and 251. Timing of implants (hutch × wean $P = 0.06$) impacted d 251-356 ADG with IMP–IMP and NOIMP-NOIMP having greater ADG than IMP-NOIMP or NOIMP-IMP. Timing of implants did not impact ($P \geq 0.18$) G:F over the course of the experiment. Hot carcass weight and ribeye area were greatest (hutch × wean $P = 0.01$) for NOIMP-NOIMP when compared to all other treatments. Marbling score was not affected ($P \geq 0.53$) by early implant regimen. Quality grades were similar across treatments ($P \geq 0.11$) with 71% grading choice. Yield grade tended ($P = 0.06$) to be greater for calves implanted during the wean phase. Implanting calves during the hutch phase lowered ($P = 0.02$) morbidity rates, whereas mortality rate was lower ($P = 0.04$) when calves were implanted in the wean phase. Overall, implanting young calves with Ralgro irrespective of the phase in which it was administered did reduce final BW but did not negatively impact morbidity rate or carcass characteristics.

Key words: carcass, growth, Holstein calves, implants, steers, Zeranol
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INTRODUCTION

Management of newborn Holstein calves can be challenging if one considers the labor and expense of handfeeding individual animals in hutches up to 90 d. What is more, calves are weaned into smaller pens around 90 d of age and placed in a wean pen until approximately 170 d of age. During these two important time frames are when growth and health are problematic. Therefore, producers continue to seek economical ways to boost performance. One effective and economical way to increase growth in calves is through the utilization of growth implants. Donovan et al. (1983) observed that rate of gain was increased by 10.7% when suckling Holsteins were implanted with 36 mg zeranol at birth, and a further 3% increase in growth occurred if the calves were reimplanted 84 d later. Likewise, McCarthy et al. (2016) reported that Holstein calves provided an implant at approximately 2 d of age had greater DMI, ADG, and a tendency for greater G:F than non-implanted calves. Likewise, Pritchard et al. (2015) reported a 10 kg increase in weaning weights

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when beef calves were implanted during the suckling phase. Typically, Holstein calves enter the feedlot at 170 d of age and are placed on an aggressive implant program and may receive up to three implants during the finishing phase. What is not known is if implanting dairy beef steers prior to the feedlot phase, specifically during the nursing phase (hutch phase) or during the weaning phase just prior to entry into the feedlot will have an impact on growth throughout the calf's lifetime. Therefore, we hypothesized that early administration of a growth implant on 1 d old Holstein calves will increase ADG and feed efficiency without negatively impacting overall health and carcass characteristics. The objectives were to determine the effects of 36 mg of Zeranol administered to Holstein calves at 1 or 92 d of age or a combination thereof on growth performance, health, and carcass characteristics.

MATERIALS AND METHODS

All procedures were conducted in accordance to the rules of the New Mexico State University Institutional Animal Care and Use Committee. Male Holstein calves (n = 1248; initial BW 41.2 ± 0.2 kg) 1 to 2 d of age were purchased and transported to Reynolds Creek Calf Ranch in Melba, Idaho. Calves arrived at the calf ranch over a 6-wk period between March 18, 2015, and April 22, 2015, and were blocked by arrival date (6 loads).

Animals and Management

All calves had ad-libitum access to water upon arrival and throughout the trial, and all cattle were processed within 24 h of arrival. On d 0, calves were individually weighed, tagged with an electronic identification tag, given a visual ear tag, vaccinated with Vision CD-T with Spur (Merck Animal Health, Madison, NJ), and castrated if needed at 35 DOF (equal number of castrated calves per block and treatment). The experiment was a completely randomized block design with a 2 × 2 factorial arrangement of treatments. The trial was segmented into 3 feeding periods including a hutch phase (d 0-92); wean phase (d 92-169), and feedlot phase (d 169-502). At the initiation of the trial (d 0), Holstein steer calves were randomly assigned to 1 of 4 treatments (n = 312 per treatment) equally distributed within each of the 6 blocks with treatments being: 1) calves received an implant at the beginning of the hutch phase on d 0 and received an implant at the beginning of the wean phase on d 92 (**IMP-IMP**), 2) received an implant on d 0 only (**IMP-NOIMP**), 3) received an implant on d 92 only (**NOIMP-IMP**), or 4) did not receive any implants in either the hutch or wean phases (**NOIMP-NOIMP**). Calf performance during the hutch phase was reported by McCarthy et al. (2016). Upon weaning (d 92), calves were moved from individual hutches to group pens. Calves were provided ad libitum access to a grain starter (17.2% CP, 4% fat and NEm 0.47 Mcal/kg and NEg 0.33 Mcal/kg), which was the same starter diet provided during the hutch phase. The amount of feed offered was adjusted using daily visual estimates of unconsumed feed remaining in the bunk. At the end of the weaning period (d 110), calves were then provided ad libitum access to a grower diet (14.5% CP, 2.75% Fat and NEm 0.44 Mcal/kg and NEg 0.30 Mcal/kg) for 82 d and then transitioned to a finishing ration (13.33% CP, 6.43% Fat and NEm 0.46 Mcal/kg and NEg 0.32 Mcal/kg). The amount of feed offered was adjusted using daily visual estimates of unconsumed feed amounts remaining in the bunk.

At the end of the wean phase (d 169), all treatments were implanted with 36 mg zeranol (Ralgro; Merck Animal Health), then on d 251, 80 mg trenbolone acetate (TBA) + 16 mg estradiol (Revalor-IS; Merck Animal Health) was administered to all calves, and the final implant (40 mg estradiol + 200 mg TBA; Revalor-XS (Merck Animal Health) was administered on d 356. Individual BW were collected on d 0, 92, 110, 169, 251, and at harvest.

Health Monitoring

Throughout the trial, the on-site veterinarian monitored health. Calves were checked for abscesses that may have occurred due to the implant procedure. Animals were deemed to be "sick", based on subjective criteria such as general appearance and attitude, gauntness, reluctance to move, etc., and then diagnosed and treated as per the written treatment protocols provided by the consulting veterinarian. The treatment events including treatment date, the presumptive diagnosis, drug(s) administered, and dose(s) were recorded. Chronic animals were determined by the animal's inability to perform once multiple treatments were administered.

Harvest and Carcass Data Collection

Steers were harvested in groups according to blocks, which had been designated upon arrival (load 1-6). The harvest schedule started August 1, 2016, to August 22, 2016, and took place every consecutive Monday for the respective 4 wk. The remaining two loads were sent on September 19, 2016, and September 26, 2016. Harvest date was based on days on feed and visual size of the steers. Final weights were recorded 1

d before slaughter. Steers in each harvest group were transported approximately 531 km to Washington Beef LLC in Toppenish, WA where they were harvested under plant procedures. Identity of each carcass was maintained using individual animal EID that was scanned to match animal and trolley number. To ensure that if errors with reading the EID occurred, trained personnel recorded ear tag numbers to track kill sequence. Hot carcass weights were obtained at slaughter.

Approximately 24 h postmortem, carcasses that had been ribbed (between the 12th and 13th ribs) went through camera grading using the VBG 2000 grading system (E + V Technology, Oranienburg, Germany; Emerson et al., 2013) to determine the factors used for USDA yield grades (fat thickness, ribeye area, and hot carcass weight) and USDA quality grades were recorded (USDA, 2016). Marbling scores were obtained for both sides of each carcass and the greater of the two marbling scores were used to assign a grade (Emerson et al., 2013). Marbling scores were assigned to each carcass using the following continuous scale: 300 = Traces, 400 = Slight, 500 = Small, 600 = Modest, 700 = Moderate, and 800 = Slightly Abundant. Backfat was obtained for both sides of each carcass and the lesser of the two scores were used to assign backfat. Dressing percentages were calculated using hot carcass weight and final weights (5% pencil shrink).

Statistical Analysis

Data were analyzed as a completely randomized block design with load serving as the block. Experimental unit was calf for all body weight measures, whereas feed intake and efficiency used pen as the experimental units. All data were analyzed using the PROC MIXED procedure of SAS (9.3; SAS Inst. Inc., Cary, NY) with hutch implant, wean implant, block, and hutch × wean interaction as the fixed effects. Least square means were compared using the Tukey test with $P < 0.05$ being considered significant and levels of $P < 0.10$ referred to as tendencies. All data are presented on a deads-out basis.

RESULTS AND DISCUSSION

Growth

Wean Phase (*d 92-169*). Wean phase performance data is presented in Table 1. Calf performance during the hutch phase was reported previously by McCarthy et al. (2016) with calves receiving implants on d-0 of the hutch period having greater BW than non-implanted calves on d 92 (weaning). Pritchard et al. (2015) reported that beef calves had greater weaning weights when implanted at approximately 45 d of age. In the wean phase; however, there were no treatment interactions or main effect differences ($P \geq 0.14$) detected for DMI. Likewise, ADG during the wean phase did not differ ($P \geq 0.13$). Furthermore, G:F was not impacted ($P \geq 0.18$) by early implanting strategy. Implanting calves in either the hutch or wean phase did not increase ($P \geq 0.14$) d 169 BW. This agrees with Mader et al. (1994) who reported that preweaning implants did not influence postweaning gains.

Feedlot Phase (d 169-Final). Steers were finished in accordance to feedlot protocol, such that all treatment groups received three implants during the finishing phase. Following administration of the first feedlot implant (Ralgro), no differences in treatments ($P \geq 0.14$) were observed for d 251 BW. There were no treatment interactions ($P = 0.59$) for d 169-251 DMI. However, implanting in the hutch resulted in greater ($P = 0.03$) d 169-251 DMI. There were no treatment interactions ($P = 0.23$) noted for d 169-251 ADG. Interestingly, implanting calves during the hutch phase continued to impact ADG out to d 169-251 ($P = 0.01$) and this growth advantage tended ($P = 0.06$) to continue out to d 356. Nevertheless, G:F was not impacted by early implanting strategy ($P \geq 0.18$) for the remainder of the experiment.

Following the second feedlot implant (Revalor-IS) on d 251, a hutch × wean interaction ($P = 0.02$) was observed for d 356 BW, such that IMP-IMP were heavier than NOIMP-IMP; additionally, IMP-NOIMP and NOIMP-NOIMP were intermediate and equal to IMP-IMP and NOIMP-IMP. Dry matter intake was not influenced by implant treatments ($P \geq 0.20$) between d 251 and 356. Timing of implants tended to impact d 251-356 ADG (hutch × wean $P = 0.06$) with IMP–IMP and NOIMP-NOIMP having greater ADG than IMP-NOIMP or NOIMP-IMP.

A treatment interaction occurred for final BW ($P < 0.01$) due to IMP-IMP and NOIMP-NOIMP steers having greater final BW compared to other 2 treatments and NOIMP-NOIMP being greater than IMP-IMP. There were no treatment interactions for DMI for d 356-final ($P = 0.45$). However, DMI during the final feeding period tended ($P = 0.08$) to be greater for calves not implanted during the wean phase. This increase in DMI translated to greater ADG for calves not implanted during the wean phase ($P = 0.03$). No treatment interactions ($P = 0.56$) were observed for ADG for the final feeding period (d 356-final), which was greater for calves that were not implanted during the hutch phase ($P < 0.01$). Samber et al. (1996) observed that neither ADG nor G:F differed among groups that received implants. Once cattle entered the feedlot

TABLE 1. Growth performance, health, and carcass characteristics of Holstein steers implanted with Ralgro

	Treatment[1]							
Hutch	IMP		NO IMP			*P*-value		
Wean	IMP	NO IMP	IMP	NO IMP	SE	Hutch	Wean	Hutch × Wean
Performance								
Weight, kg								
d 0	41.3	40.9	41.3	41.4	0.2	0.30		
d 92[2]	99.7	98.4	95.2	95.8	0.7	<0.01		
d 110[3]	118.0	116.4	114.2	113.7	0.9	<0.01	0.24	0.55
d 169[4]	181.0	178.8	178.5	177.1	1.5	0.14	0.23	0.75
d 251[5]	294.5	290.9	289.8	290.3	1.8	0.14	0.38	0.25
d 356[6]	439.5[a]	431.6[ab]	428.7[b]	431.9[ab]	2.6	0.04	0.32	0.02
Final[7]	658[a]	653[b]	656[ac]	667[d]	0.4	<0.01	<0.01	<0.01
DMI, kg/d								
d 92-169	3.34	3.22	3.26	3.34	0.07	0.78	0.83	0.14
d 169-251	5.51	5.41	5.28	5.27	0.08	0.03	0.48	0.59
d 251-356	7.54	7.62	7.52	7.75	0.11	0.65	0.20	0.53
d 356-final	9.09	9.30	9.27	9.77	0.20	0.12	0.08	0.45
ADG, kg/d[8]								
d 92-169	1.05	1.04	1.08	1.06	0.013	0.17	0.13	0.61
d 169-251	1.35	1.34	1.29	1.31	0.01	0.01	0.71	0.23
d 251-356	1.36	1.34	1.32	1.34	0.01	0.06	0.99	0.06
d 356-final	1.49	1.52	1.56	1.61	0.02	< 0.01	0.03	0.56
G:F kg gain/kg feed								
d 92-169	0.333	0.339	0.344	0.329	0.009	0.99	0.59	0.27
d 169-251	0.246	0.248	0.245	0.250	0.007	0.97	0.65	0.83
d 251-356	0.190	0.181	0.186	0.184	0.004	0.86	0.18	0.37
d 356-final	0.159	0.158	0.162	0.159	0.03	0.54	0.54	0.70
Carcass								
No. Pens	6	6	6	6				
Final SBW[9], kg	624	621	624	634	0.40	<0.01	<0.01	<0.01
HCW, kg	378[ab]	375[ab]	373[a]	381[b]	2.35	0.83	0.19	0.01
Dressing %[10]	60.69	60.66	59.99	60.40	0.37	0.19	0.59	0.54
Backfat, cm	0.752	0.726	0.714	0.706	0.007	0.08	0.35	0.63
Ribeye area, cm	12.29[a]	12.32[ac]	12.18[a]	12.59[bc]	0.08	0.34	0.01	0.01
YG[11]	2.86	2.80	2.82	2.74	0.04	0.15	0.06	0.80
Marbling[12]	550	546	548	544	7	0.78	0.53	0.99
USDA Choice, %	71.8	74.2	67.4	70.9	2.9	0.16	0.28	0.84
Health								
Mean d to first pull	28.51	27.58	28.22	26.96	2.34	0.84	0.63	0.94
Morbidity								
%	92.9	92.0	94.9	96.5	1.3	0.02	0.79	0.34
Mortality								
%	9.62	5.45	6.09	4.48	1.38	0.11	0.04	0.35
Removals[13]								
%	9.94	8.65	7.37	5.13	1.51	0.04	0.24	0.75
Total removals and mortality								
%	19.55	14.10	13.46	9.62	1.97	0.01	0.02	0.68

[a–c]Means within row that do not have a common superscript differ, P < 0.05.

[1]Treatment: Calves were randomly assigned to one of four treatments. Treatment group Hutch: did or did not receive Ralgro implant going into the hutch on d 0 (IMP or NOIMP, respectively); Wean: did or did not receive Ralgro implant at weaning on d 92 (IMP or NOIMP, respectively). All calves were then implanted on d 169, 251, and 356 with Ralgro, Revalor-IS, and Revalor-XS, respectively.

[2]All calves were individually weighed and treatments groups IMP-IMP: n = 284 & NOIMP-IMP: n = 289 received Ralgro implant on d 92. Treatment IMP-NOIMP: n = 287 and NOIMP-NOIMP: n = 300 did not receive an implant on d 92.

[3]All calves were individually weighed; treatment groups IMP-IMP: n = 282, IMP-NOIMP: n = 284, NOIMP-IMP: n = 289 and NOIMP-NOIMP: n = 297.

[4]All calves were individually weighed and received Ralgro implant: IMP-IMP: n = 266, IMP-NOIMP: n = 279, NOIMP-IMP: n = 280, and NOIMP-NOIMP: n = 289.

[5]All calves were individually weighed and received Revalor-IS implant: IMP-IMP: n = 255, IMP-NOIMP: n = 264, NOIMP-IMP: n = 262, and NOIMP-NOIMP: n = 267.

[6]All calves were individually weighed and received Revalor-XS implant: IMP-IMP: n = 262, IMP-NOIMP: n = 274, NOIMP-IMP: n = 276, and NOIMP-NOIMP: n = 287.

[7]Average unshrunk final weight of lot and treatment groups: IMP-IMP: n = 251, IMP-NOIMP: n = 268, NOIMP-IMP: n = 270, and NOIMP-NOIMP: n = 282.

8ADG was calculated on an individual animal basis, since individual weights were recorded.

[9]SBW = shrunk body weight.

[10]Dressing percentages were calculated using hot carcass weight and final weights (5 % pencil shrink).

[11]YG = calculated yield grade.

[12]Marbling Score: 300 = Traces, 400 = Slight, 500 = Small, 600 = Modest, 700 = Moderate, 800 = Slightly Abundant.

[13]Removals = chronic animals were determined by the animal's inability to perform once multiple treatments were administered, thus removed.

phase herein, variation in performance was noted with proportional increases in DMI and ADG, which precluded the observation of G:F differences. Scheffler et al. (2003) reported that ADG in Holstein steers that received 2 or 3 implants did not differ but were both greater than steers receiving one or no implant during a 291 finishing period. Although speculation, the greater final BW and ADG for NOIMP-NOIMP may have been due to compensatory growth response to implants once these animals entered the feedlot phase.

Carcass Characteristics

Carcass measurements are presented in Table 1. There was a hutch × wean interaction ($P = 0.01$) for HCW because NOIMP-NOIMP was greater than NOIMP-IMP; whereas IMP-IMP and IMP-NOIMP did not differ. In contrast, no differences ($P = 0.19$) in dressing percentage were detected between implant regimens. Perry et al. (1991) utilized a combination implant consisting of trenbolone acetate and estradiol in Holstein steers and reported increases in HCW of implanted steers with no differences in dressing percentage. Likewise, Simms et al. (1988) reported similar dressing percentages for steers implanted with multiple zeranol implants through a suckling, growing and finishing phase study. There was a hutch × wean interaction ($P = 0.01$) for ribeye area due to the differences between NOIMP-IMP and NOIMP-NOIMP being greater than the difference between IMP-IMP and IMP-NOIMP. Calves implanted during the wean phase had smaller ribeye area ($P = 0.01$) than the non-implanted groups. Not implanting calves during the wean phase tended to lower yield grade ($P = 0.06$). Treatments did not impact final marbling score ($P \geq 0.53$). There were no treatment differences ($P \geq 0.16$) reported for quality grades with all treatments grading 67% choice or above. Scheffler et al. (2003) reported a slight reduction in marbling score but no difference in steers grading choice when Holstein steers were provided 0, 1, 2, or 3 implants during the finishing phase.

Health

No differences ($P \geq 0.63$) were observed for mean days to first pull. The mean d to first pull was 27.8 ± 2.34 d from initial implant at receiving until steers were sent to harvest. There were no treatment interactions ($P \geq 0.34$) for morbidity rate, mortality, or removals. Implanting calves during the hutch phase lowered ($P = 0.02$) morbidity rates, whereas mortality rate was lower ($P = 0.04$) when calves were implanted in the wean phase. The average morbidity rate throughout the trial was 94.1%. Over the course of 502 DOF (or 523 for blocks 5 and 6), 177 animals were removed from the data set due to mortality (n = 80) and removals (n = 97). Overall, implanting at a very early age increased the percentage of calves that died or were removed irrespective of when implant was administered (hutch $P = 0.01$; wean $P = 0.02$). A greater percentage (16.8 vs. 11.5%, respectively) of calves implanted in the hutch phase were removed due to death or deemed chronic compared to those not implanted. Additionally, when implants were administered in the wean phase, 16.5% of calves in IMP-IMP and NOIMP-IMP either died or were removed. Munson et al. (2012) found that delaying implants to 45 d post-arrival to the feedlot cattle tended to decrease morbidity. Conversely, Richeson et al. (2015) reported no differences in morbidity rates in calves provided implants compared to non-implant controls.

In conclusion, the dairy beef industry is continually looking for ways to decrease costs of production. However, when we look at lifetime performance, no differences in intakes or G:F were observed in calves implanted in either the hutch, wean phase, or both thereof. From a carcass standpoint, treatment regimens had similar YG, REA, but had lower HCW compared to steers not implanted in the hutch or wean phases. Delaying implanting until feedlot entry (NOIMP-NOIMP) appeared to perform as well as calves that received a combination of implants at a very young age. Furthermore, implanting in the hutch or wean time periods was detrimental to performance and health as evidenced by lower final BW, ADG, HCW, and health parameters.

IMPLICATIONS

In general, implanting calves at a very early age does not appear to be advantageous to a producer who maintains ownership through harvest. Likewise, dairy beef producers that maintain ownership only until entry into the feedlot would likely not see significant BW or feed efficiency advantages with implanting. Therefore, administration of implants during the hutch or wean phases of dairy beef production systems do not seem to provide any advantage from a growth, health, or carcass perspective.

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Effects of zinc source and dietary concentration on zinc status, growth performance, and wool characteristics in developing rams[1]

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ABSTRACT: The objectives of this study were to evaluate the effects of dietary zinc source and concentration on Zn status, growth performance, and wool characteristics in developing rams. We hypothesized greater dietary Zn concentrations, and a more bioavailable chemical form would result in greater serum Zn concentrations, growth performance and efficiency and wool characteristics. Forty-four Targhee rams (14 mo of age; 68 ± 18 kg BW) were used in an 84-d completely randomized design, and were fed one of three pelleted dietary treatments: 1) a control diet without fortified zinc (CON; n = 15); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp; n = 14); and 3) a diet fortified with $ZnSO_4$ ($ZnSO_4$; n = 15). Growth and wool traits measured throughout the course of the study were ADG, DMI, G:F, BW, loin muscle depth (LMD), back fat (BF), wool staple length (SL), and average fiber diameter (AFD). Jugular venous samples were collected from each ram at four time periods to quantify serum Zn concentrations. Data were analyzed using the MIXED and GLIMMIX procedures of SAS for repeated and single trait measurements. There were no differences in DMI ($P = 0.18$), BW ($P = 0.45$), LMD ($P = 0.48$), BF ($P = 0.47$), and AFD ($P = 0.9$) among treatment groups. $ZnSO_4$ had greater ($P \leq 0.03$) serum Zn concentrations compared to ZnAA and CON treatments. Rams consuming ZnAA had greater ($P \leq 0.03$) ADG than $ZnSO_4$ and CON. There tended to be differences among groups for G:F ($P = 0.06$), with ZnAA being greater than $ZnSO_4$ and CON. SL was greater ($P < 0.001$) in the $ZnSO_4$ treatment group and tended to be longer ($P = 0.06$) in ZnAA treatment group compared to CON. These results indicate that the source and concentration of a Zn supplement appears to improve ram development, specifically ADG, serum Zn concentrations, SL, with a tendency to improve G:F. Although zinc retention and metabolic pathways of zinc metabolism were not investigated, results indicate that greater dietary zinc concentrations could be beneficial to ram development. This evidence can be utilized to make sound management decisions when accounting for minerals with developing rams in Montana and other northern range lands.

Key words: Bioavailability, ram, trace minerals, zinc
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INTRODUCTION

Western sheep production systems rely largely on rangeland plant communities as the primary feed source. This reliance on the rangeland plant community could lead to mineral deficiencies, which may limit the productivity of livestock operations. Mineral concentrations in forages are highly variable across rangelands (Mathis and Sawyer, 2004) with influential factors such as soil geochemistry (Smith et al., 2014) and forage stage of maturity (Jones and Tracy, 2015). Numerous studies have suggested that the chemical form of a mineral source plays an important role in bioavailability; generally with organic sources being more bioavailable than inorganic sources (Rojas et al., 1995; Spears, 2003). A survey conducted to quantify serum Zn concentrations in Montana ram lamb populations indicated that approximately 14% of ranches sampled were

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categorized as being deficient and 52% marginally deficient in Zn (Page et al., 2016).

Zinc is the second most abundant trace mineral in the body with important functions involved in reproduction (Kumar et al., 2006), gene expression (Berg, 1990), immune function (Spears and Weiss, 2008), and wool growth in sheep (White et al., 1994). Cholecystokinin, an appetite regulating hormone is thought to be affected by the gene expression properties of zinc which in turn could affect growth rate (Blanchard and Cousins, 1996). Subclinical deficiencies in Zn could be more frequent than other trace minerals because the body does not sequester large amounts of available Zn in any one organ. (NRC, 2007; Herdt and Hoff, 2011). Optimal concentrations of dietary Zn are not well understood, and with such high tolerance to dietary Zn in most mammals, there is potential for higher supplementation levels than the recommended concentrations for sheep (NRC, 2007). The objective of the present study was to quantify the effects that dietary zinc source and concentration have on developing ram zinc status, growth performance, and wool characteristics.

MATERIALS AND METHODS

Animals and Diets

Experimental procedures described herein were approved by the Agriculture Animal Care and Use Committees of Montana State University (#2016-AA09). All animals used in this study were provided by the Montana Agricultural Experiment Station, and the study was conducted at the Fort Ellis Research Station at Montana State University in Bozeman, MT.

Forty-four purebred Targhee rams (14 mo of age; 68±18 kg BW) were utilized in an 84 d completely randomized design. Rams were stratified by BW, serum Zn concentrations and allocated to one of three pelleted dietary treatments: 1) control diet without fortified Zn (**CON**; n = 15; Table 1); 2) a diet fortified with a Zn amino acid complex (**ZnAA**, Zinpro Corp; n = 14); and 3) a diet fortified with $ZnSO_4$ (**$ZnSO_4$**; n = 15). The basal diet was formulated to meet 100% of nutrient and Zn requirements of developing rams (24 mg of Zn/kg; NRC, 2007). Zinc dietary treatments were formulated to provide 300% of Zn (72 mg Zn/kg) and 100% of nutrient requirements. Ram treatment groups were randomly assigned to drylot pens (6.4 × 21.9 m) with approximately one third of the pen covered by a three-sided barn. Rams were fitted with an electronic identification tag and each pen was equipped with two GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to monitor individual intake. Rams had *ad libitum* access to a NaCl source, feed, and water. Water source was sampled prior to study and analyzed by a commercial laboratory for livestock suitability (Midwest Labs, Omaha, NE). Rams were denied access to a complete free choice granulized mineral premix d -50 to d 0 of the study to normalize trace mineral status. Rams were fed a dry hay diet from d -16 to d 0 to deplete circulating Zn concentration in the blood.

TABLE 1. Chemical and nutrient composition of dietary treatments

	Dietary Treatments[1]		
Item	CON	ZnAA	$ZnSO_4$
Ingredient, %			
Alfalfa, DHY-17	37.47	37.43	37.46
Corn, ground	30	30	30
Soybean hulls	15	15	15
Malt sprouts	10	10	10
Molasses, cane	4	4	4
Calcium carbonate	1.35	1.35	1.35
Ammonium chloride	1	1	1
Mineral premix	1.18	1.18	1.18
Nutrient Composition[2]			
DM, %	90.41	90.41	90.41
CP, %[3]	17.6	16.8	17.4
NDF, %	30.72	30.71	30.72
ADF, %	20.72	20.71	20.72
Ash, %	7.65	7.65	7.67
Mineral Composition[2]			
Ca, %	1.24	1.24	1.24
P, %	0.35	0.35	0.35
K, %	1.5	1.5	1.5
S, %	0.19	0.19	0.19
Mg, %	0.21	0.21	0.21
Na, %	0.39	0.39	0.39
Fe, mg/kg	237.37	237.2	237.31
Mn, mg/kg	89.14	89.13	89.14
Cu, mg/kg	7.87	7.86	7.87
Zn, mg/kg[3]	47.5	95.5	91.5
I, mg/kg	0.9	0.9	0.9

[1]Dietary treatments: 1) control diet without fortified zinc (CON); 2) a diet fortified with a Zn amino acid complex (ZnAA); and 3) a diet fortified with $ZnSO_4$.

[2]Calculated concentration in diets.

[3]Analyzed concentration in diets.

Feed Intake, Growth, Ultrasound, and Wool Data

Rams were adapted to the GrowSafe system for 16 d prior to the start of the study. Individual intake data was recorded by the GrowSafe system. Elevated platforms were used to modify GrowSafe beef cattle stanchions and feed bunks (0.79 × 1 m) for sheep. Feed in-

take was monitored daily and additional feed was added to the bunk as needed. Rams were weighed on consecutive days on d -1 and 0, 27 and 28, 55 and 56, and 83 and 84. For the consecutive days at the beginning and end of the study rams were fasted 12 h before weights were recorded. On d 27, 28, 55, and 56 rams were given free access to feed prior to BW being recorded.

Ultrasonic measurements of loin muscle depth (**LMD**) and back fat (**BF**) were taken on d 0, 28, 56, and 84 of the study by the same National Sheep Improvement Program certified ultrasound technician using a real-time ultrasound device (ibex-pro, E.I. Medical Imaging, Loveland, CO). Before each ultrasound image was captured the area was shorn and vegetable oil was used as a conductive medium. Images were captured between the 12th and 13th rib with an 8-5 MHz 66 mm linear array transducer.

Wool side samples were collected from rams on d 0 and d 84, and wool staple length (**SL**) growth over the 84-d study was measured at 5 locations and averaged for each ram. Wool side samples were prepared and analyzed for fiber diameter (**AFD**) and other wool traits by the Montana State Wool Lab utilizing the OFDA 2000 optical scanning device.

Blood Collection and Analysis

Blood samples were collected via jugular venipuncture into 13 × 100 mm trace mineral royal blue top vacutainer tubes (Covidien, Mansfield, MA) without additives for blood serum analysis. The first blood sample was obtained on d -16 of the study for the purpose of stratifying groups by serum Zn status. Blood samples were then collected on d 28, 56, and 84 of the study. Blood samples were kept on ice and allowed to clot for approximately 4 hours (Herdt and Hoff, 2011) and then centrifuged at 2700 × *g* for 30 min at 4ºC. Serum was decanted into two aliquots in 12 × 75 mm plastic culture tubes and stored at -20˚C for later analysis. Serum Zn concentrations were determined by a commercial laboratory (Michigan State University Diagnostic Center for Population and Animal Health, East Lansing) using an ionized coupled plasma mass spectrometry method (Wahlen et al., 2005).

Statistical Analysis

Data were analyzed as a completely randomized design with individual ram as experimental unit. Growth performance and intake data were analyzed as repeated measures using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). The model included the fixed effects of treatment, day, and their interaction, and the random effect of ram nested within treatment. An autoregressive covariance structure with heterogeneous variance across day was assumed, which was found to be the most parsimonious using Akaike's information criteria. Wool traits measured at the end of the trial were analyzed using the GLIMMIX procedure with the fixed effect of treatment and the wool measurement at the start of the trial was fit as a linear covariate. Data are presented as least squares means of main effects and differences were considered significant at $P \leq 0.05$ and as a tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

Effects of Zn source and dietary concentration on ADG, DMI, G:F, and BW are presented in Tables 2 and 3. There was no difference ($P = 0.18$) among treatments for DMI. Overall, there was no difference ($P = 0.45$) among treatments in BW. Rams consuming ZnAA had greater ($P \leq 0.03$) ADG than $ZnSO_4$ and CON rams. Similar results were found in lambs supplemented with Zn-methionine and ZnO, with a tendency of Zn-methionine to increase growth performance (Spears, 1989). There tended to be differences among groups for G:F ($P = 0.06$) with ZnAA being greater than $ZnSO_4$ and CON. Although Zn deficiency is less of a clinical problem in ruminant animals (Herdt and Hoff, 2011), some of the initial signs of deficiency include poor feed intake and reduced growth rates (Herdt and Hoff, 2011). Zinc's effects on growth and intake performance may be in part modulated by its relationship with cholecystokinin. Cholecystokinin secretion is increased in Zn-deficient intestinal tissues and serves roles in endocrine and neurocrine functions regulating gall bladder contraction, pancreatic secretion, gastric emptying, and satiety mechanisms (Blanchard and Cousins, 1996).

Effects of Zn on ultrasound measurements of LMD and BF are presented in Table 3. There were no differences in LMD ($P = 0.48$) and BF ($P = 0.47$) among treatments.

Wool traits for rams measured in the study are presented in Table 3. Average wool fiber diameter (AFD) did not differ ($P = 0.96$) among treatments regardless of Zn source or concentration. Staple length (SL) was greatest ($P < 0.001$) in rams consuming fortified $ZnSO_4$ diets compared to CON; whereas ZnAA tended ($P = 0.06$) to have longer staple length over the 84-d study than CON. Zinc is a major constituent in wool (NRC, 2007), and Zn plays a critical role in the keratinization process through structural and regulatory factors (Tomlinson et al., 2004), which could provide a reasonable explanation for rams consuming greater concentrations of Zn, despite different sources, tended to have longer SL. Zinc deficiencies reduce wool growth and impair keratinization in wool

TABLE 2. Effects of dietary Zn source and period on the performance of rams, carcass traits, serum Zn concentrations, and wool traits

	Treatment[1]					Period				
Item	CON	ZnAA	$ZnSO_4$	SEM[2]	*P* – value	d 0 to 28	d 29 to 56	d 57 to 84	SEM[2]	*P*–value
ADG, kg/d	0.33[b]	0.40[a]	0.34[b]	0.18	0.03	0.44[a]	0.40[a]	0.23[b]	0.20	<0.001
DMI, kg/d	3.11	3.32	3.18	0.81	0.18	2.81[a]	3.43[b]	3.37[b]	0.57	<0.001
G:F	0.109[b]	0.124[a]	0.109[b]	0.005	0.06	0.158[a]	0.115[b]	0.068[c]	0.005	<0.001

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.

[2]Greatest SEM presented (n = 15).

[a–c]LS means, within a row, lacking common superscripts differ ($P < 0.05$).

TABLE 3. Effects of dietary Zn source and day on the performance of rams, carcass traits, serum Zn concentrations, and wool traits

	Treatment[1]					Day					
Item[3]	CON	ZnAA	$ZnSO_4$	SEM[2]	*P* – value	0	28	56	84	SEM[2]	*P*–value
BW, kg	83.7	87.0	84.1	2.02	0.45	68.4[a]	80.8[b]	92.1[c]	98.4[d]	1.30	<0.001
LMD, mm	30.02	29.80	29.13	0.55	0.48	25.30[a]	28.34[b]	30.56[c]	34.41[d]	0.50	<0.001
BF, mm	4.45	4.70	4.70	0.17	0.47	2.98[a]	4.28[b]	5.25[c]	5.96[d]	0.15	<0.001
Serum Zn, μg/mL[4]	0.63[b]	0.66[b]	0.71[a]	0.16	0.002	0.54[a]	0.53[a]	0.71[b]	0.89[c]	0.02	<0.001
SL, mm	23.37[b]	25.91[b]	26.67[a]	1.02	0.003	—	—	—	—	—	—
AFD, micron	22.1	22.1	22.0	0.34	0.96	—	—	—	—	—	—

[1]Dietary treatments: 1) control diet without fortified zinc (CON; Table 1); 2) a diet fortified with a Zn amino acid complex (ZnAA, Zinpro Corp); and 3) a diet fortified with $ZnSO_4$.

[2]Greatest SEM presented (n = 15).

[3]LMD: loin muscle depth; BF: back fat; SL: wool staple length; AFD: average fiber diameter.

[4]d 0 measurements were collected d -16.

[a-d] LS means, within a row, lacking common superscripts differ ($P < 0.05$).

through a specific mechanism, perhaps involving protein synthesis (White et al., 1994).

Serum Zn concentrations were greatest ($P \leq 0.03$) in $ZnSO_4$ (Table 3; Figure 1); whereas, serum Zn concentration did not differ ($P = 0.12$) between ZnAA and CON. Zinc homeostasis is tightly regulated in the body (Herdt and Hoff, 2011), and resultant Zn tissue concentrations remain relatively constant over a wide range of Zn intakes. There is no clear site of accumulation of Zn throughout the body and Zn absorption is reduced under conditions of ample Zn intake (NRC, 2007; Herdt and Hoff, 2011), which could offer a reasonable explanation for not having observed an increase in serum Zn levels in ZnAA, with this study. In a similar study, Zn absorption did not differ between Zn sources, but retention was greater in lambs treated with Zn Methionine than with a ZnO source, indicating difference in metabolism post-absorption or tissue retention (Spears, 1989).

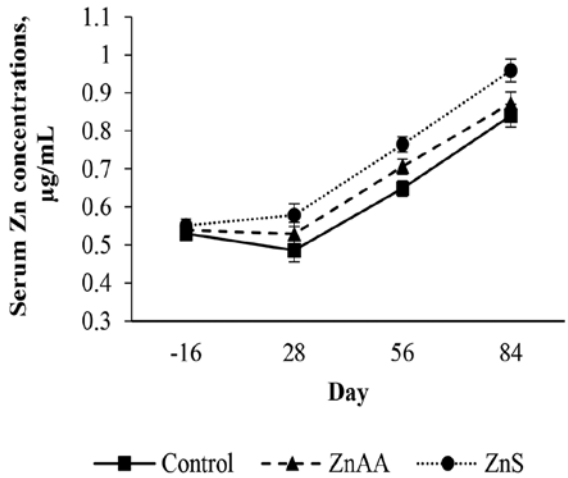

Figure 1. Effects of Zn source on serum Zn concentrations in rams. Treatment x day: $P = 0.22$; dietary treatment: $P = 0.002$; and day: $P < 0.0001$.

IMPLICATIONS

Overall, Zn source and concentration affected ADG, serum Zn concentrations, staple length, and tended to increase feed efficiency. Results indicate the beneficial effects of supranutritional Zn concentrations beyond basal dietary concentrations. Although Zn retention and metabolic pathways of Zn metabolism were not investigated, results indicate that greater dietary Zn concentrations can enhance nutritional strategies in ram develop-

ment. These findings might be especially applicable to producers developing white-face type rams for fall ram sales in the mountain west and northern plains regions.

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Supplementing a yeast-derived product to enhance productive and health responses of feeder steers

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ABSTRACT: This experiment evaluated the impacts of supplementing a yeast-derived product (Celmanax; Church & Dwight Co., Inc.; Princeton, NJ) on productive and health responses of feeder steers, and was divided into a preconditioning (d 4 to 30) and feedlot receiving phase (d 31 to 69). Eighty-four Angus × Hereford steers were weaned at approximately 7 mo of age (d -4), and maintained in a single group from d -4 to 3. On d 4, steers were allocated according to weaning BW and age to a 21-pen drylot (5 steers/pen). Pens were randomly assigned to receive: 1) no Celmanax supplementation during the experiment (n = 7), 2) supplementation with Celmanax (14 g/steer daily; as-fed) from d 14 to 69 (n = 7), or 3) supplementation with Celmanax (14 g/steer daily; as-fed) from d 31 to 69 (n = 7). Steers had free-choice access to grass-alfalfa hay and received a corn-based concentrate beginning on d 14. Celmanax was mixed daily with the concentrate. On d 30, steers were road-transported for 1,500 km (24 h). On d 31, steers returned to their original pen assignment for a 38-d feedlot receiving. Shrunk BW was recorded on d 4, 31, and 70. Feed DMI was evaluated from each pen daily (d 14 to 69). Steers were observed daily (d 4 to 69) for bovine respiratory disease (BRD) signs. Preconditioning results were analyzed by comparing pens that received (CELM) or not (CON) Celmanax during the preconditioning phase. Feedlot receiving results were analyzed by comparing pens that received Celmanax from d 14 to 69 (CELPREC), d 31 to 69 (CELRECV), or no Celmanax supplementation (CON). During preconditioning, incidence of BRD was less (P = 0.03) in CELM compared with CON steers. During feedlot receiving, ADG tended (P = 0.07) to be greater in CELPREC and CELRECV vs. CON steers. No treatment differences were detected ($P \geq 0.29$) for DMI parameters; hence, G:F also tended (P = 0.08) to be greater in CELPREC and CELRECV vs. CON steers. No further treatment differences were detected ($P \geq 0.20$) for performance, health, and blood variables during the experimental period. In summary, Celmanax supplementation reduced BRD incidence during a 30-d preconditioning. Moreover, Celmanax improved ADG and G:F during a 38-d feedlot receiving, independently if supplementation began during preconditioning or upon feedlot entry. Hence, Celmanax supplementation appears to be a nutritional strategy to enhance health parameters and receiving performance of feeder cattle.

Key words: cattle, growth, health, supplementation, yeast
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INTRODUCTION

Transport and feedlot entry are two of the most stressful events experienced by feeder cattle (Swanson and Morrow-Tesch, 2001). Upon long transportation periods and during the initial 30 d at the feedlot, cattle experience inflammatory and acute-phase responses (Cooke, 2017) known to impair their productivity and health, including susceptibility to respiratory diseases (Berry et al., 2004; Araujo et al., 2010). These inflammatory and acute-phase responses are elicited by several stress-related stimuli (Cooke, 2017) including endotoxemia caused by 1) death of rumen microbes upon feed/water deprivation during transport and 2) major dietary changes during feedlot receiving (Marques et al., 2012). Consequently, nutritional efforts to enhance gut and overall health upon transport and feedlot entry are

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warranted to optimize productivity and welfare of feeder cattle (Duff and Galyean, 2007).

One strategy to enhance gut and overall immune function during feedlot receiving is to supplement cattle with yeast-derived products, such as cultures and extracts (Cole et al., 1992; Brown and Nagaraja, 2009). Accordingly, Ponce et al. (2012) supplemented beef heifers during a 35-d receiving period with Celmanax (Church & Dwight Co., Inc.; Princeton, NJ), a commercial product containing yeast culture and enzymatically hydrolyzed yeast product. These authors reported that heifers receiving Celmanax had greater growth rates, feed intake, and reduced morbidity caused by respiratory diseases compared with non-supplemented heifers. However, Ponce et al. (2012) did not evaluate immunological and physiological responses to elucidate the biological benefits of Celmanax supplementation on cattle health and performance traits. In addition, Ponce et al. (2012) began supplementing Celmanax to heifers 1 d after feedlot arrival, which is after the critical period of stress and microbial death caused by feed/water deprivation during road transport (Marques et al., 2012).

Based on this latter rationale, we hypothesized that beginning Celmanax supplementation to feeder cattle prior to transport and feedlot entry, such as during a 30-d preconditioning period (Pritchard and Mendez, 1990), will further increase its health and performance benefits during feedlot receiving. Therefore, this experiment evaluated the impacts of Celmanax supplementation beginning at preconditioning or upon feedlot entry on performance, health and physiological variables of beef steers during preconditioning followed by a 38-d feedlot receiving.

MATERIALS AND METHODS

All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4862). This experiment was divided into a preconditioning (d 4 to 30) and feedlot receiving phase (d 31 to 69).

Cattle Diets and Management

Eighty-four Angus × Hereford steers were weaned at 7 mo of age (d -4), and maintained in a single meadow foxtail pasture for 7 d (d -4 to 3). On d 4, steers were allocated according to weaning BW and age to a 21-pen drylot (7 × 15 m; 5 steers/pen). Pens were randomly assigned to receive 1 of 3 treatments: 1) no Celmanax supplementation during the experiment (n = 7), 2) supplementation with Celmanax (14 g/steer daily) from d 14 to 69 (n = 7), or 3) supplementation with Celmanax (14 g/steer daily) from d 31 to 69 (n = 7).

TABLE 1. Ingredient composition (as-fed basis; kg/d) of concentrate offered during preconditioning (d 4 to 30) and feedlot receiving (d 31 to 69) phases 1

Item	Whole corn	Soybean meal	Mineral[2]
Preconditioning	0.64	0.23	0.05
Feedlot receiving			
A	0.91	0.36	0.05
B	2.27	0.36	0.05
C	4.10	0.55	0.05

[1]Preconditioning concentrate was offered from d 14 to 30. During feedlot receiving, A = d 31 to 36; B = d 37 to 44; and C = d 45 to 69. Steers had free-choice access to grass-alfalfa hay throughout the experimental period (d 4 to 69). Hay and concentrate were offered separately, in different sections of the feed bunk.

[2] Cattleman's Choice (Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E.

During the preconditioning phase (d 4 to 30), steers had free-choice access to grass-alfalfa hay and received a corn-based concentrate (Table 1) beginning on d 14. Celmanax was mixed daily with the concentrate. Within each pen, hay and concentrate were offered separately in different sections of the feed bunk. On d -4 and d 18, steers were vaccinated against *Clostridium* (One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea virus Types 1 and 2, and *Mannheimia haemolytica* (Bovi-Shield Gold One Shot; Zoetis).

On d 30, all steers were commingled and transported at the same time and in the same double-deck commercial livestock trailer (Legend 50' cattle liner; Barrett LLC., Purcell, OK) for 1,500 km (24 h) to simulate the stress of a long-haul (Marques et al., 2012). On d 31, steers returned to their original pen assignment for a 38-d feedlot receiving phase. During feedlot receiving, steers had free-choice access to grass-alfalfa hay and received a corn-based concentrate (Table 1), with Celmanax also mixed daily with the concentrate. Within each pen, hay and concentrate were offered separately in different sections of the feed bunk.

Sampling

Steer shrunk BW (after 16 h of water and feed withdrawal) was recorded on d 4 and 70, and on d 31 (after transport) for ADG calculation. Feed DMI was evaluated daily from d 14 to 69 by weighing and collecting samples of the offered and non-consumed feed from each pen. All samples were dried for 96 h

at 50°C in forced-air ovens for DM calculation. Feed efficiency during the feedlot receiving phase was calculated according to total DMI and BW gain of each pen. Steers were observed daily (d 4 to 69) for sickness, particularly bloat (Meyer and Bartley, 1972) and bovine respiratory disease (**BRD**; Berry et al., 2004). Cattle received (i.m.) 0.1 mL/kg of BW of Hexasol LA Solution (Norbrook® Inc. USA; Overland Park, KS) when BRD signs were observed, or 60 mL (oral drench, mixed with 500 ml of water) of Therabloat (Zoetis) when bloat was detected.

Blood samples were collected on d 14, 30, 31, 33, 35, 40, 45, 54, and 69. Samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing no additive or containing 158 USP units of freeze-dried sodium heparin for serum and plasma collection, respectively. After collection, all blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. Serum samples collected from d 14 to 54 were analyzed for NEFA concentrations (colorimetric kit HR Series NEFA – 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA). Plasma samples collected from d 14 to 54 were analyzed for cortisol (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA) and haptoglobin (Cooke and Arthington, 2013) concentrations. Plasma samples collected on d 14, 30, 54, and 69 were analyzed for IGF-I concentrations (Immulite 1000; Siemens Medical Solutions Diagnostics). The intra- and interassay CV were, respectively, 1.7 and 6.8% for NEFA, and 3.0 and 4.5% for haptoglobin. Plasma IGF-I and cortisol were analyzed within single assays, and the intra-assay CV were, respectively, 2.7, and 1.4%.

Statistical analysis

Pen was considered the experimental unit. Results from the preconditioning phase were analyzed by comparing pens that received (**CELM**) or not (**CON**) Celmanax during preconditioning. Results from the feedlot receiving phase were analyzed by comparing pens that received Celmanax from d 14 to 69 (**CELPREC**), d 31 to 69 (**CELRECV**), or no Celmanax supplementation during the experiment (**CON**). In addition, treatment effects during feedlot receiving were compared using pre-planned single-df orthogonal contrasts (CELPREC and CELRECV vs. CON; CELPREC vs. CELRECV).

Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.), and Satterthwaite approximation to determine the denominator df for tests of fixed effects. All data were analyzed using pen(treatment) and steer(pen) as random variables, but for DMI and G:F that used pen(treatment) as random variable. Model statement for BW, ADG, G:F, and morbidity and mortality rates within each phase contained the effects of treatment. Model statement for DMI, cumulative BRD incidence, and blood variables contained the effects of treatment, day, and the resultant interaction, in addition to results from d 14 as independent covariate for blood variables only. The specified term for the repeated statements was day, with pen(treatment) as subject for DMI and steer(pen) as subject for blood variables and cumulative BRD incidence. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. All results are reported as least square means, but for blood variables that are reported as covariately adjusted least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant, or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

During the preconditioning phase, no treatment differences were detected ($P \geq 0.20$) for BW, ADG, and intake parameters (Table 2). However, incidence of respiratory disease was less ($P = 0.03$) in CELM steers compared with CON steers (Table 2). It is important to note that all cases of BRD signs during the preconditioning phase were observed from d 16 to 30 (treatment × day interaction, $P < 0.01$; Fig. 1); hence, after treatments began to be administered. These results indicate that supplementing Celmanax during preconditioning did not enhance steers performance traits, but eliminated the incidence of BRD typically observed in recently-weaned cattle (Taylor et al., 2010). Although the effects of yeast products on cattle immunity is not clearly established, yeast components such as β-glucan are positively associated with proliferation and responsiveness of T-cells to antigens or cytokines (Nocek et al., 2011). Accordingly, others have reported similar outcomes in cattle supplemented with Celmanax during feedlot receiving (Ponce et al., 2012) or upon bovine rhinotracheitis virus challenge (Cole et al., 1992).

During the feedlot receiving phase, ADG tended ($P = 0.07$) to be greater in CELPREC and CELRECV vs. CON steers, and was similar ($P = 0.89$) between CELPREC and CELRECV steers (Table 3). No treat-

TABLE 2. Performance and health parameters during the preconditioning phase (d 4 to 30) in steers receiving a concentrate containing (CELM; n = 7) or not (CON; n = 14) 14 g/steer daily of Celmanax (Church & Dwight Co., Inc.; Princeton, NJ) from d 14 to 30

Item	CON	CELM	SEM	*P* =
Growth parameters[1]				
Initial BW, kg	230	232	3	0.60
Post-transport BW, kg	242	245	3	0.52
ADG, kg/d	0.46	0.52	0.05	0.41
DMI parameters[2]				
Hay, kg/d	5.27	5.41	0.10	0.27
Concentrate, kg/d	0.44	0.50	0.04	0.26
Total, kg/d	5.55	5.77	0.11	0.20
Health parameters[3]				
Morbidity, %	16.0	0.0	4.9	0.03
Bloat, %	0.0	0.0	-	-
Respiratory, %	16.0	0.0	4.9	0.03
Mortality, %	0.0	0.0	-	-

[1]Steer shrunk BW was recorded after 16 h of water and feed withdrawal on d 4 (initial BW), and after road transport (1,500 km for 24 h) on d 31.

[2]Feed intake was recorded daily from d 14 to 30 by measuring offer and refusals from each pen.

[3]Steers were observed daily (d 4 to 69) for sickness, particularly bloat (Meyer and Bartley, 1972) and respiratory disease (Berry et al., 2004).

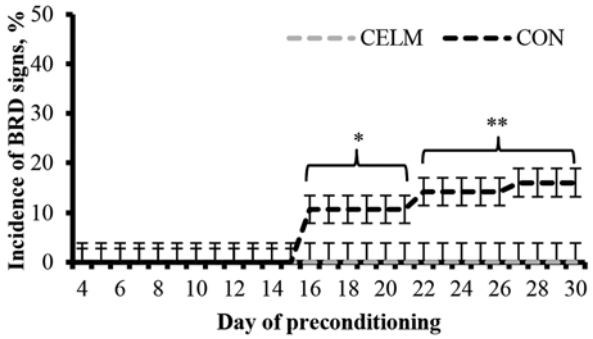

Figure 1. Incidence of bovine respiratory disease (BRD) signs, according to Berry et al. (2004), during the preconditioning phase (d 4 to 30) in steers receiving a concentrate containing (CELM; n = 7) or not (CON; n = 14) 14 g/steer daily of Celmanax (Church & Dwight Co., Inc.; Princeton, NJ) from d 14 to 30. A treatment × day interaction was detected ($P < 0.01$). Within day; * $P = 0.03$, ** $P < 0.01$.

ment differences were detected ($P \geq 0.29$) for DMI parameters (Table 3). Hence, G:F also tended ($P = 0.08$) to be greater in CELPREC and CELRECV vs. CON steers, and was similar ($P = 0.54$) between CELPREC and CELRECV steers (Table 3). However, treatment differences detected for ADG were not sufficient to impact ($P \geq 0.44$) steer final receiving BW (Table 3), and no treatment differences were detected ($P \geq 0.22$) for morbidity and mortality parameters (Table 3).

No treatment differences were detected ($P \geq 0.27$) for concentrations of plasma cortisol, plasma haptoglobin, plasma IGF-I, and serum NEFA (Table 4). As expected based on experimental design, day effects were detected for all blood variables (Fig. 2). Plasma cortisol and haptoglobin concentrations transiently increased ($P \leq 0.05$) across all treatments after transport, demonstrating that steers experienced a neuroendocrine and subsequent acute-phase protein response elicited by transport and feedlot entry (Marques et al., 2012; Cooke, 2017). Serum NEFA concentrations also transiently increased across all treatments after transport, which can be associated to water and nutrient deprivation during transport and the cortisol-induced lipolysis (Marques et al., 2012). Plasma IGF-I concentrations increased across all treatments during feedlot receiving, mainly due to increased nutrient intake (Table 1) and growth (Table 3) during this phase (Elsasser et al., 1989).

Supporting our hypothesis and results from Ponce et al. (2012), Celmanax supplementation improved feedlot receiving ADG and G:F. These outcomes were independent if Celmanax supplementation began during preconditioning or upon feedlot entry. Hence, beginning Celmanax supplementation during preconditioning, to allow cattle to consume and adapt to the product prior to the stress of road transport and feedlot entry, failed to further increase receiving performance and health despite eliminating BRD incidence during preconditioning. Moreover, Celmanax supplementation did not influence circulating concentrations of variables associated with stress, inflammation, and nutritional status in feeder cattle (Cooke, 2017). Perhaps Celmanax supplementation improves performance and health of feeder cattle, as reported herein and by Ponce et al. (2012), without modulating systemic physiological responses; although further research is warranted to support this rationale.

It is important to note, however, that BRD incidence during the feedlot receiving phase was not as prevalent compared with values from research conducted at commercial receiving yards (Snowder et al., 2006; Marques et al., 2016). This outcome can be associated with the fact that while steers were subjected to the stress of road transportation (Cooke, 2017), they returned to the same facility with the same pen members, and were not exposed to cattle from other sources in a novel environment (Step et al., 2008). Hence, research with cattle exposed to a high-stress commercial feedlot scenario is also warranted to further investigate the physiological, immune, and performance effects of Celmanax supplementation, starting either during preconditioning or upon feedlot entry.

TABLE 3. Performance and health parameters during a 38-d feedlot receiving period (d 31 to 69) in steers receiving 14 g/d of Celmanax (Church & Dwight Co., Inc.; Princeton, NJ) during preconditioning and feedlot receiving (d 14 to 69; CELPREC; n = 7), during feedlot receiving only (d 31 to 69; CELRECV; n = 7), or not receiving Celmanax during the experiment (d 4 to 69; CON; n = 7)

					Single df contrasts[1]	
Item	CON	CELPREC	CELRECV	SEM	1	2
Growth parameters[2]						
Final BW, kg	302	309	304	4	0.47	0.44
ADG, kg/d	1.51	1.61	1.62	0.048	0.07	0.89
DMI parameters[3]						
Hay, kg/d	4.13	4.30	4.26	0.14	0.41	0.85
Concentrate, kg/d	2.94	3.00	2.93	0.09	0.83	0.60
Total, kg/d	7.07	7.29	7.19	0.13	0.29	0.57
G:F,[4] g of BW/kg DMI	219	227	231	4	0.08	0.54
Health parameters[5]						
Morbidity	10.7	14.2	17.8	7.3	0.56	0.73
Bloat, %	10.7	10.7	17.8	7.3	0.69	0.50
Respiratory, %	0.0	3.5	0.0	2.0	0.48	0.22
Mortality, %	3.5	3.5	0.0	2.9	0.62	0.39

[1]Single-df orthogonal contrasts: 1 = CON vs. CELPREC and CELRECV, and 2 = CELPREC vs. CELRECV.

[2]Steer shrunk BW was recorded after road transport (1,500 km for 24 h) on d 31, and after 16 h of water and feed withdrawal on d 70 (final BW).

[3]Feed intake was recorded daily from d 31 to 69 by measuring offer and refusals from each pen.

[4]Calculated according to total DMI and BW gain of each pen

[5]Steers were observed daily (d 4 to 69) for sickness, particularly bloat (Meyer and Bartley, 1972) and bovine respiratory disease (Berry et al., 2004).

TABLE 4. Concentrations of plasma cortisol, plasma haptoglobin, plasma IGF-I, and serum NEFA in steers receiving 14 g/d of Celmanax (Church & Dwight Co., Inc.; Princeton, NJ) during preconditioning and feedlot receiving (d 14 to 69; CELPREC; n = 7), during feedlot receiving only (d 31 to 69; CELRECV; n = 7), or not receiving Celmanax during the experiment (d 4 to 69; CON; n = 7)[1]

					Single df contrasts[2]	
Item	CON	CELPREC	CELRECV	SEM	1	2
Plasma cortisol, ng/mL	30.3	28.0	31.4	2.1	0.82	0.27
Plasma haptoglobin, mg/mL	0.258	0.233	0.221	0.034	0.46	0.82
Plasma IGF-I, ng/mL	198	203	207	7	0.41	0.73
Serum NEFA, μEq/L	0.288	0.283	0.283	0.009	0.65	0.94

[1]Blood samples were collected on d 14, 30, 31, 33, 35, 40, 45, 54, and 69. Serum samples collected from d 14 to 54 were analyzed for NEFA concentrations. Plasma samples collected from d 30 to 54 were analyzed for cortisol and haptoglobin concentrations. Plasma samples collected on d 14, 30, 54, and 69 were analyzed for IGF-I concentrations. Results from d 14 were used as independent covariate for each respective analysis; hence, values reported are covariately-adjusted least square means.

[2] Single-df orthogonal contrasts: 1 = CON vs. CELPREC and CELRECV, and 2 = CELPREC vs. CELRECV.

IMPLICATIONS

Celmanax supplementation reduced incidence of respiratory disease but did not enhance performance of recently-weaned steers during a 30-d preconditioning. Moreover, Celmanax supplementation improved growth and feed efficiency during a 38-d feedlot receiving, independently if Celmanax supplementation began during preconditioning or upon feedlot entry. Hence, Celmanax supplementation appears to be a nutritional strategy to enhance health parameters and receiving performance of feeder cattle.

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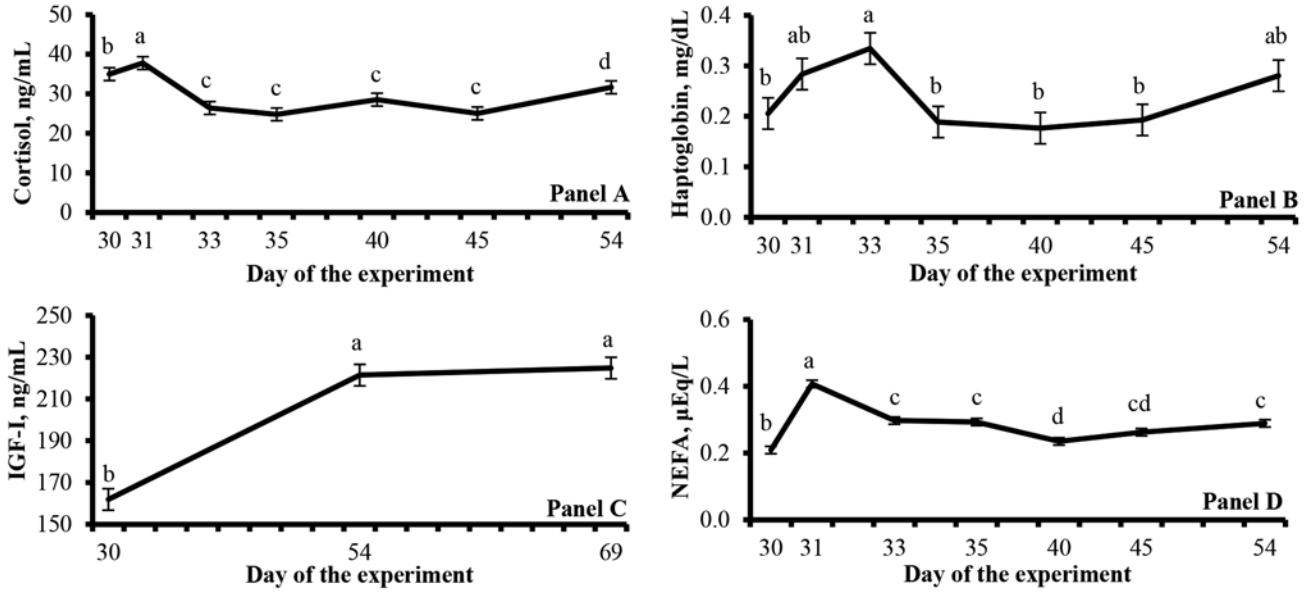

Figure 2. Concentrations of plasma cortisol (Panel A), plasma haptoglobin (Panel B), plasma IGF-I (Panel C), and serum NEFA (Panel D) during the experiment, On d 30, steers were loaded into a livestock trailer and transported for 1,500 km (24 h), and assigned to a 38-d feedlot receiving (d 31 to 69). Day effects were detected for all variables ($P < 0.01$). Within variable, days with different letters (a-d) differ ($P < 0.05$).

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Supplementing an immunomodulatory feed ingredient to modulate thermoregulation, physiological, and production responses in lactating dairy cows under heat stress conditions

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ABSTRACT: This study compared vaginal temperature, physiologic, and productive parameters in lactating dairy cows supplemented or not with Omnigen-AF (Phibro Animal Health, Teaneck, NJ). Thirty-two lactating, primiparous (n = 16) and multiparous (n = 16) pregnant Holstein × Gir cows were ranked by parity, days in milk (DIM), BW and BCS, and assigned to receive (SUPP; n = 16) or not (CON; n = 16) Omnigen-AF at 56 g/cow daily (as-fed basis). Cows were maintained in a single drylot pen with ad libitum access to water and a TMR, and milked twice daily. Cows received Omnigen-AF mixed with 200 g of corn (as-fed basis) after the daily morning milking through self-locking head gates, whereas CON cows concurrently received 56 g of kaolin mixed with 200 g of corn. For DMI evaluation, cows from both treatments were divided in 4 groups of 8 cows each, and allocated to 8 individual feeding stations for 3 d. Intake was evaluated 4 times per group from d 1 to 56. From d -6 to 0, d 15 to 28, and d 43 to 56, cow vaginal temperature was recorded hourly. Environmental temperature humidity index (THI) was recorded hourly from d 15 to 28 and d 43 to 56. Cow BW was recorded on d -6 and 56, individual milk production was recorded daily from d -6 to 56, and milk samples were analyzed for somatic cell count (SCC) and milk components. Blood samples were collected on d -6, -3, 0, 9, 15, 18, 21, 24, 27, 36, 45, 48, 51, 54, and 56. Results from d -6 to 0 were included as an independent covariate in each respective analysis. Environmental THI was 74.2 ± 0.5 and cows were exposed to THI > 68 for 633 h within a total of 672 h of evaluation. Cows assigned to CON had greater ($P \leq 0.05$) vaginal temperature on d 28, 43, 45 and from d 48 to 55 (by 0.38 to 0.52%), as well as greater ($P \leq 0.05$) mean SCC (by 97%) and serum haptoglobin concentrations (by 89%) compared with SUPP cows. Cows assigned to SUPP had greater ($P \leq 0.10$) mean DMI (by 7%), BCS on d 56 (by 11%), and mean serum insulin concentrations (by 35%) compared with CON cows. Hence, SUPP ameliorated hyperthermia, improved nutritional status, and modulated systemic and mammary gland immune parameters in lactating dairy cows exposed to heat stress conditions.

Key words: heat stress, lactating cow, milk production, Omnigen-AF, temperature
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INTRODUCTION

Heat stress is one of the major challenges to dairy production systems in subtropical and tropical environments (West, 2003). St-Pierre et al. (2003) estimated that heat stress costs the US dairy industry approximately $900 million annually, whereas decreased milk yield is a critical contributor to this outcome. Hyperthermia is known to impact milk production by reducing voluntary DMI (West, 1994), but also impairs metabolic and physiological processes required for optimal cattle productivity and welfare (West, 2003; Collier et al., 2008; Rhoads et al., 2009). Hence, management strategies that alleviate the incidence of heat stress in dairy cows are warranted to optimize profitability in dairy production systems (West, 2003).

Omnigen-AF is a patented proprietary branded product recently shown to improve milk production and innate immunity parameters in transition dairy cows (Brandão et al., 2016). However, sup-

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plementing this ingredient has also been associated with decreased hyperthermia in cattle subjected to heat stress conditions. Brandão et al. (2016) reported that supplemented cows had reduced mean vaginal temperature compared with non-supplemented cohorts during periods with temperature humidity index (**THI**; Zimbleman et al., 2009) > 68. Supplementing this ingredient also decreased rectal temperature and respiration rates in lactating (Hall et al., 2014) and non-lactating (Fabris et al., 2016) dairy cows exposed to elevated thermal and humidity load. Collectively, these results imply that this ingredient impacts thermoregulation of dairy cows under heat stress conditions. Yet, research is still warranted to verify its potential thermoregulatory capabilities (Brandão et al., 2016), including supplementation to lactating dairy cows in a production system with elevated incidence of THI > 68. Therefore, this experiment evaluated the effects of Omnigen-AF supplementation on vaginal temperature, metabolic, physiologic, and productive parameters of lactating dairy cows during the summer months in a tropical environment.

MATERIALS AND METHODS

This experiment was conducted from December 2015 to February 2016 at the São Paulo State University – Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. The animals utilized were cared for in accordance with protocols reviewed and approved by the São Paulo State University - Animal Ethics Committee.

Animals and diets

Thirty-two lactating, primiparous (n = 16) and multiparous (n = 16), pregnant Holstein × Gir cows (initial mean ± SE; BW = 517 ± 11 kg, BCS = 3.06 ± 0.06, DIM = 167 ± 9 d, 1.6 ± 0.2 parities) were ranked by parity, DIM, BW, and BCS (Wildman et al., 1982) and assigned to receive (**SUPP**; n = 16) or not (**CON**; n = 16) 56 g/cow daily (as-fed basis) of Omnigen-AF (Phibro Animal Health, Teaneck, NJ) from d 1 to 56.

During the experimental period (d -6 to 56), cows were maintained in a single drylot pen with ad libitum access to water and a TMR. The drylot pen had no available shade or cooling system. The TMR consisted (DM basis) of 58.9% corn silage and 41.1% concentrate and was formulated to yield 25 kg of milk /day. Cows were milked twice daily (0600 and 1700 h) from d -6 to 56.

From d 1 to 56, SUPP cows individually received 56 g of Omnigen-AF (SUPP) or kaolin (CON) mixed with 200 g of finely-ground corn (as-fed basis), through self-locking head gates that had no shade, but contained a sprinkler + fan cooling system.

Sampling

Intake parameters. Cows from both treatments were randomly divided in 4 groups of 8 cows each that were allocated to 8 individual feeding stations for 3 d. During this period, cows from the selected group continued to have ad libitum access to water and TMR, and received treatments as previously described. The TMR DMI was evaluated daily from each feeding station. At the end of the 3-d period, cows returned to the drylot pen and another group was assigned to the individual feeding stations, in a manner that DMI was evaluated 4 times/group during the experimental period.

Temperature parameters. From d -6 to 0, d 15 to 28, and d 43 to 56, cows were fitted intravaginally with a thermometer (iButton temperature loggers DS1922L, Maxim Integrated, San Jose, CA) attached to a controlled internal drug-releasing device (CIDR, Zoetis, São Paulo, SP, Brazil) that did not contain hormones. Cow vaginal temperature was recorded hourly during the 6-d (d -6 to 0; **PR1**) or each 14-d periods (d 15 to 28, **PR2**; d 43 to 56, **PR3**). Environmental temperature, relative humidity, and THI were recorded hourly using 2 HOBO Water Temp Pro V2 data loggers (Onset Company, Bourne, MA) from d 15 to 28 and d 43 to 56. Data were summarized (mean ± SE) as daily THI, and hourly THI across days (Fig. 1a and 1b). During PR1, PR2, and PR3, cows were also evaluated for total hours with temperature ≥ 39.1°C, % of hours with temperature ≥ 39.1°C, and mean vaginal temperature when THI was >68 (Zimbleman et al., 2009) as previously reported by Brandão et al. (2016).

Cow productive parameters. Individual milk production was recorded daily from d -6 to 56. Milk samples were collected weekly from each cow. Milk samples were analyzed for SCC via flow cytometry, and concentrations of fat and total solids via infrared spectrometry. Daily milk yield was adjusted to fat-corrected (**FCM**) or total solids (**TS**)-corrected milk based on milk concentrations of fat and total solids, respectively, of the concurrent week. Cows were evaluated for BW and BCS on d -6 and 56.

Physiological and metabolic parameters. Blood samples were collected for serum harvest every three days. Samples were stored at -20°C until analysis.

All samples were analyzed for serum concentrations of glucose, NEFA, haptoglobin, IGF-I, cortisol and insulin using the procedures described by Brandão et al. (2016). Insulin to glucose ratio (**I:G**) was determined by dividing insulin and glucose concentrations within each sampling time (Bernhard et al., 2012). The intra-

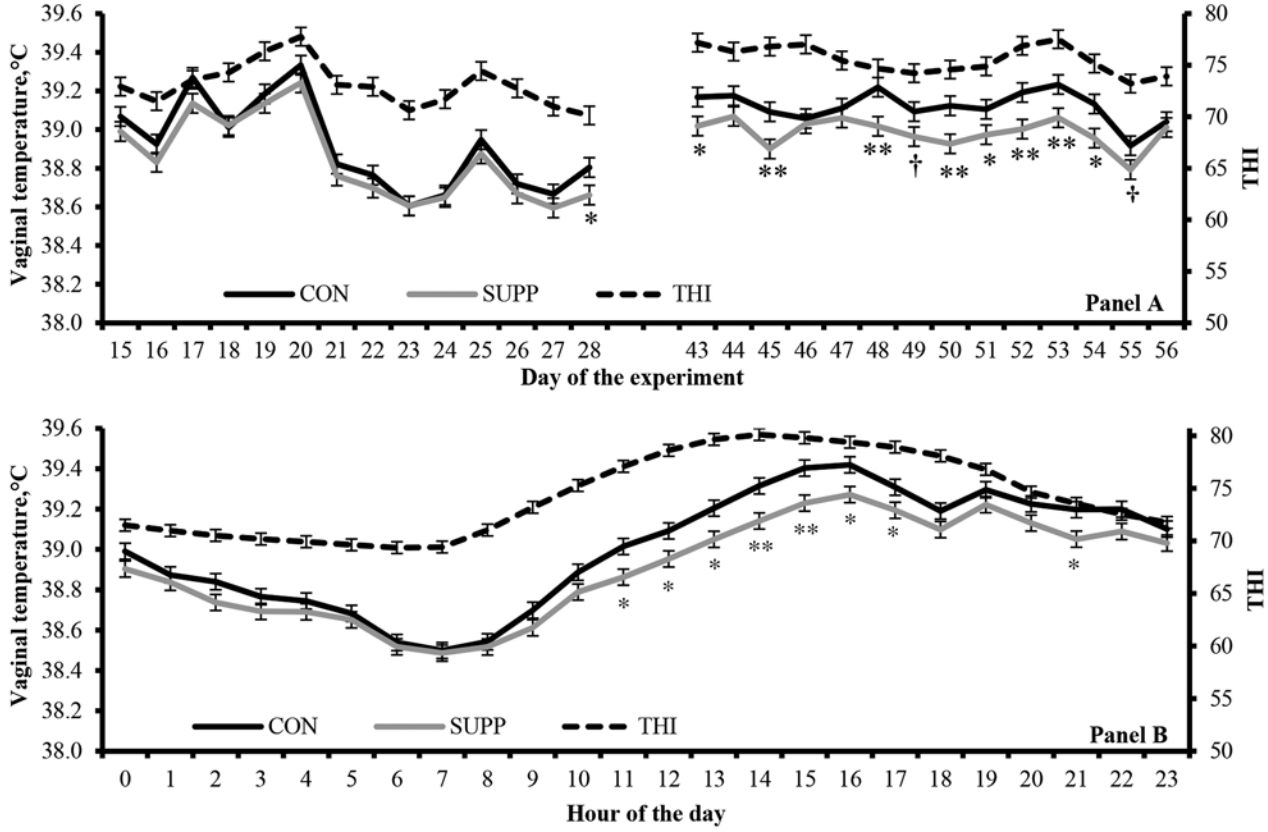

Figure 1. Environmental THI and vaginal temperature in dairy cows supplemented with an immunomodulatory feed ingredient (Omnigen-AF; Phibro Animal Health, Teaneck, NJ; SUPP; n = 16) or not (CON; n = 16) from d 1 to 56. Treatment × day (panel A) and treatment × hour (panel B) interactions were detected ($P \leq 0.01$). Within day or hour, ** = $P \leq 0.01$; * = $P \leq 0.05$, † = $P \leq 0.01$.

and interassay CV were, respectively, 3.1 and 4.3% for glucose, 4.3 and 3.5% for NEFA, and 2.0 and 4.8% for haptoglobin. Serum IGF-I, cortisol, and insulin concentration were analyzed within single assays, and the intra-assay CV were, respectively, 3.1, 1.4, and 0.4%.

Statistical analysis

Quantitative and binary data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc.; version 9.3) using cow as the experimental unit. The model statement used for analysis of BW and BCS change, as well as initial and final BCS and BW during the experiment contained the effects of treatment, party, and the resultant interaction, with cow(treatment × parity) as random variable. The model statement used for analysis of milk yield, milk constituents, and serum variables contained the effects of treatment, day, parity, and all resultant interactions. Cow(treatment × parity) was used as random variable, whereas the specified term for the repeated statements was day with cow(treatment × parity) as subject. The model statement used for analysis of DMI contained the effects of treatment, day, group, parity, and all resultant interactions. Cow(group × treatment × parity) was used as random variable, whereas the specified term for the repeated statements was day(group) with cow(group × treatment × parity) as subject. The model statement used for analysis of vaginal temperature contained the effects of treatment, period (PR2 or PR3), day, hour, parity, and all resultant interactions. Cow(group × period × parity) was used as random variable, whereas the specified term for the repeated statements was hour(day × period) with cow(group × period × parity × day) as subject. The model statement for vaginal temperature variables when THI was > 68 was analyzed using treatment, parity, period (PR2 or PR3), and all resultant interactions, with cow(treatment × parity) as random variable. All vaginal temperature analyses contained averaged results from samples or observations collected from PR1 as independent covariates. The covariance structure utilized for all repeated statements was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported as least square means, or covariately adjusted means for analyses that included a covariate, and separated using LSD. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected that contained the effects of treatment.

TABLE 1. Vaginal temperature parameters of dairy cows supplemented with an immunomodulatory feed ingredient (Omnigen-AF; Phibro Animal Health, Teaneck, NJ; SUPP; n = 16) or not (CON; n = 16) during heat stress conditions (THI > 68; Zimbleman et al., 2009) for 56 d

Item	SUPP	CON	SEM	*P* =
Time with temperature ≥ 39.1°C, h				
Period 2 (d 15 to 28)	91.3	112.3	11.4	0.21
Period 3 (d 43 to 56)	128.3	167.5	11.4	0.02
% hours with temperature ≥ 39.1°C, %				
Period 2 (d 15 to 28)	30.5	37.4	3.7	0.21
Period 3 (d 43 to 56)	43.2	54.8	3.7	0.03
Mean temperature, °C				
Period 2 (d 15 to 28)	38.88	38.91	0.05	0.62
Period 3 (d 43 to 56)	39.01	39.12	0.05	0.10

RESULTS

Temperature parameters

While environmental THI was being recorded, mean (± SE) THI was 74.2 ± 0.5, and cows were exposed to THI > 68 for 633 h within a total of 672 h. Moreover, mean THI during PR2 was less ($P \leq 0.01$) compared with mean THI of PR3 (73.1 vs. 75.4, respectively; SEM = 0.5).

A treatment × day interaction was detected ($P \leq 0.01$; Fig. 1a) for vaginal temperature, given that CON cows had greater (by 0.38 to 0.52%) temperatures on d 28, 43, 45 ($P \leq 0.05$), and from d 48 to 55 of the experiment ($P \leq 0.09$) compared with SUPP cows. A treatment × hour interaction was also detected ($P = 0.01$), as vaginal temperatures were greater in CON vs. SUPP cows (by 0.29 to 0.44%) from 1100 to 1700 h ($P \leq 0.04$) and at 2100 h ($P \leq 0.01$; Fig. 1b).

Treatment × period interactions were detected ($P \leq 0.04$) for vaginal temperature parameters when THI was > 68 (Table 1). More specifically, no treatment differences were detected ($P \geq 0.21$) for these parameters during PR2. However, CON cows had more ($P = 0.02$) time with vaginal temperature ≥ 39.1 (by 30%), greater ($P = 0.03$) percentage of time with vaginal temperature ≥ 39.1 (by 27%), and tended ($P = 0.10$) to have greater mean vaginal temperature (by 0.28%) compared with SUPP cohorts when THI was > 68 during PR3 (Table 1).

Intake and cow productive parameters

During the experimental period, there was a statistical tendency ($P = 0.10$) for SUPP cows to have greater DMI (by 7%) and BW change compared with CON cows. Cow BCS change was also greater ($P = 0.03$) in SUPP vs. CON cows. Final BCS was greater ($P = 0.05$; by 11%) in SUPP vs. CON cows, although final BW did not differ ($P = 0.97$) among treatments (Table 2).

TABLE 2. Body weight and BCS of dairy cows supplemented with an immunomodulatory feed ingredient (Omnigen-AF; Phibro Animal Health, Teaneck, NJ; SUPP; n = 16) or not (CON; n = 16)

Item	SUPP	CON	SEM	*P* =
Daily DMI, kg/d[1]	18.0	16.8	0.5	0.10
Initial BW (d -6), kg	512	522	15	0.65
Final BW (d 56), kg	519	518	14	0.97
BW change, kg	7.2	-3.4	4.5	0.10
Initial BCS (d -6)	3.10	3.01	0.08	0.42
Final BCS (d 56)	3.34	3.01	0.12	0.05
BCS change	0.23	0.00	0.07	0.03

TABLE 3. Milk production parameters of dairy cows supplemented with an immunomodulatory feed ingredient (Omnigen-AF; Phibro Animal Health, Teaneck, NJ; SUPP; n = 16) or not (CON; n = 16) for 56 d[1]

Item	SUPP	CON	SEM	*P* =
Milk yield, kg/d	21.0	20.3	0.4	0.26
Fat-corrected milk, kg/d	28.9	29.2	0.97	0.82
Solids-corrected milk, kg/d	22.4	22.4	0.7	0.97
SCC, cells/mL	384	759	103	0.01

[1]Individual milk production was recorded daily from d -6 to 56. Milk samples were collected on d -6, 0, 7, 14, 21, 28, 35, 42, 49, and 56 from each cow.

No treatment differences were detected for ($P \geq 0.26$) milk production, FCM or TS-corrected milk. Somatic cell count was greater ($P = 0.01$; by 97%) in CON vs. SUPP cows (Table 3).

Physiologic and metabolic parameters

During the experimental period, there was a statistical tendency ($P = 0.09$) for SUPP cows to have greater mean serum insulin concentrations (by 35%) compared with CON cows. Mean serum haptoglobin concentration was greater ($P = 0.05$; by 89%) in CON vs. SUPP cows. No treatment differences were detected ($P \geq 0.16$) for serum concentrations of cortisol, glucose, IGF-I, NEFA, and I:G (Table 4).

DISCUSSION

According to the THI values observed herein (Fig. 1a and 1b), cows were exposed to heat stress conditions during the vast majority of the experimental period (Zimbleman et al., 2009). Supporting our hypothesis SUPP reduced vaginal temperatures in lactating dairy

TABLE 4. Serum variables of dairy cows supplemented with an immunomodulatory feed ingredient (Omnigen-AF; Phibro Animal Health, NJ; SUPP; n = 16) or not (CON; n = 16) for 56 d[1]

Item	SUPP	CON	SEM	*P* =
Cortisol, ng/mL	8.32	8.31	0.61	0.99
Glucose, mg/dL	63.0	64.2	0.8	0.30
Haptoglobin, μg/mL	123	233	39	0.05
IGF-I, ng/mL	161	166	19	0.87
Insulin, pmol/L	3.73	2.75	0.43	0.09
NEFA, μEq/L	0.245	0.259	0.011	0.38
Insulin:glucose ratio	0.059	0.043	0.007	0.16

[1]Blood samples were collected on d -6, -3, 0, 9, 15, 18, 21, 24, 27, 36, 45, 48, 51, 54, and 56 of the experiment.

cows exposed to heat stress conditions (Fig. 1a; Table 1). Treatment differences for vaginal temperature were more evident during PR3 (d 43 to 56) of temperature evaluation (Fig. 1a), which is corroborated by the treatment × period interaction detected for vaginal temperature parameters recorded when THI > 68 (Table 1). This outcome can be attributed to the greater THI observed during PR3 vs. PR2 (Fig. 1a), suggesting that the THI threshold in which SUPP ameliorated hyperthermia in dairy cows may be greater than the THI value associated with heat stress (68; Zimbleman et al., 2009). Nevertheless, no treatment differences were detected for vaginal temperatures during days within PR2 (i.e. d 18 to 21) when THI was equivalent to levels observed during PR3. This may also suggest that there is a delay between SUPP feeding and its thermoregulatory effects, although research is warranted to validate both assumptions.

Similar to our findings, Hall et al. (2014) reported that supplementation with Omnigen-AF reduced rectal temperatures in lactating cows exposed to heat stress conditions. Yet, the same result was not observed when cows were exposed to a thermoneutral environment. This suggests that this feed ingredient reduced hyperthermia in cows during heat stress episodes, but not when cows are exposed to thermoneutral environments. Accordingly, the treatment × hour interaction detected herein for vaginal temperature (Fig, 1b) demonstrate that SUPP ameliorated the hyperthermia caused by the circadian increase in THI, but not when THI were at its lowest levels. Figure 1b also suggest that there is 1-h lag between circadian increase or decrease in THI with respective changes in vaginal temperature, which may help explaining why vaginal temperatures differed between CON and SUPP cows when THI was ≥ 75. Nevertheless, the exact mechanism by which this feed ingredient modulates thermoregulation remains unclear, and requires further investigation.

Treatment differences detected for DMI (Table 2), however, denote the productive implications of decreased vaginal temperature in SUPP vs. CON cows, and corroborates the rationale that hyperthermia directly impacts voluntary feed intake (West, 1994; Rhoads et al., 2009). Moreover, DMI is positively associated with metabolic heat increment (Kadzere et al., 2002), whereas the opposing treatment differences in vaginal temperature and DMI suggests enhanced thermoregulatory ability of SUPP cows during heat stress conditions. However, DMI differences among treatments were not sufficient to impact milk yield parameters (Table 3). One could speculate that cows in this experiment were already producing their maximum milk yield (Leiva et al., 2015) and the additional nutrients consumed by SUPP cows were converted into BCS, which would explain treatment differences detected for this latter variable (Table 2), that is also supported by the greater insulin serum concentrations detected in SUPP cows (Table 4; Butler, 2003). Besides, similar serum concentrations of glucose, NEFA, and IGF-I between SUPP and CON cows reported herein (Table 4) corroborate the similar milk yield among treatments (Rhoads et al., 2009).

During heat stress, cows experience a heightened adrenocortical function, culminating in increased circulating concentrations of cortisol (Wise et al., 1988). In the present experiment, however, serum cortisol concentrations were similar between SUPP and CON cows despite treatment differences in vaginal temperature. It can be speculated that treatment differences in vaginal temperature were not sufficient to impact adrenocortical function. The time of blood collection (06h00) and the stress reaction elicited by handling the cows may also have influenced serum cortisol concentrations.

Similar to cortisol, haptoglobin is also a key component of the bovine acute-phase and stress response (Cooke and Bohnert, 2011). Supplementation with this ingredient has been shown to increase serum haptoglobin concentration in dairy cattle following a pathogen challenge such as LPS, but not in transition dairy cows during routine management (Brandão et al., 2016). Therefore, treatment differences detected herein for serum haptoglobin concentrations provides evidence that CON cows experienced heightened adrenocortical function due to increased hyperthermia, which resulted in greater serum haptoglobin concentrations compared with SUPP cohorts.

Treatment differences detected herein (Table 3) for milk SCC were also reported by others, corroborating the immunological benefits attributed to this feed ingredient (Wang et al., 2009). In addition, mammary gland infection and inflammation that lead to increased milk SCC also yield an acute-phase reaction, which in

turn increase circulating concentrations of haptoglobin (Murata et al., 2004). Therefore, treatment differences in SCC also contributed to the greater serum haptoglobin concentrations in CON vs. SUPP cows.

IMPLICATIONS

Offering Omnigen-AF to lactating dairy cows exposed to heat stress conditions reduced hyperthermia. In turn, cows receiving Omnigen-AF had greater intake but similar milk yield, resulting in greater body condition score gain compared with non-supplemented cows. Moreover, cows receiving Omnigen-AF had less milk somatic cell count and serum haptoglobin concentrations compared with non-supplemented cows, which can be attributed to immunomodulatory effects of this ingredient. Collectively, this experiment demonstrated that Omnigen-AF supplementation appears to be an effective approach to ameliorate hyperthermia and enhance welfare and productivity in dairy systems with incidence of heat stress conditions.

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Change of season impacts ruminal fermentation and microbiome in heifers grazing native range

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ABSTRACT: A study was conducted with the objective to determine the rumen microbiome and fermentation parameters of three ruminally cannulated Angus-crossbred heifers (232 ± 12 kg BW) on dormant native rangelands over one year. We hypothesize that as seasons of the year change so will diet quality, rumen fermentation end products and bacterial populations present in the rumen. Heifers were maintained on native range and supplemented 20% CP range cube. Ruminal fluid was collected from April 2016 to February 2017 approximately every two to three months for ruminal ammonia, VFA, and bacterial population composition. Amplification and sequencing of the V4 hypervariable region of the 16S rRNA gene using the Illumina MiSeq. *Bacteroidetes* (43.4%) and *Firmicutes* (40.5%) were the major phyla throughout the sampling period. *Firmicutes* differed by day with the greatest population occuring February (45.8 ± 2.2%), while *Bacteroidetes* did not differ by sampling day. The predominant genera throughout study remained *Prevotella* (17.4%) and *Bacteroides* (9.3%) and did not differ by sampling day ($P > 0.15$). Total VFA concentrations ($P < 0.01$) and acetate:propionate ($P < 0.01$) increased for the duration of the study. Ammonia levels differed by sampling day with the greatest level occurring during February (17.0 ± 1.4%). These data shows the rumen fermentation parameters and microbiome are influenced by the varying diet quality that occurs on native rangelands.

Key words: microbiome, rangeland, ruminal ammonia
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INTRODUCTION

When ruminants graze native rangelands they are exposed to a diverse population of grasses, forbs and shrubs, and seasonal changes occur in the botanical composition of diets selected by grazing animals (Funk et al., 1987). The extent of fiber digestibility in the rumen is controlled by plant structure and composition which regulate bacterial access to nutrients, nature of population densities of fiber-digesting microorganisms, and microbial factors that control adhesion and hydrolysis. Ammonia and VFA are principal end products of ruminal bacteria fermentation. The VFAs produced in the rumen diffuse through the rumen epithelium and represent a key source of energy for the host animal (Russell et al., 1992). Ammonia is a product of ruminal protein degradation, is absorbed through the rumen wall and detoxified in the liver to urea, which can be recycled back to the rumen or excreted through urine (Russell et al., 1992). Ruminal ammonia can be utilized for microbial crude protein (MCP) synthesis (Bergmann, 1990; Webb, 1990) which can account for up to 65 % of MP for the animal.

The composition of the rumen microbiome is influenced by the quality of ingredients in the diet of a ruminant (Firkins and Yu, 2015). Many studies in the literature identify ruminal bacteria population composition but there is limited datasets available that combine microbiome information with ruminal fermentation end product production. Additionally, many rumen microbiome studies are short in duration and do not involve grazing ruminants on native rangelands. The objective of this study to characterize ruminal microbiome and fermentation end product responses to change in season and diet quality

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using cannulated heifers on native range. We hypothesize that as seasons of the year change so will diet quality, rumen fermentation end products and bacterial populations present in the rumen.

MATERIALS AND METHODS

Animals and Treatments. New Mexico State University Institutional Animal Care and Use committee approved all experimental procedures associated with this study (NMSU IACUC number: 2015-039). Three ruminally cannulated Angus-crossbred heifers (232 $\pm$ 12 kg BW) were used to determine effect of changing diet quality on rumen fermentation and bacterial populations over a year. Heifers were maintained on native range (average nutrient values: 5.44% CP, 72.64% NDF, and 44.82% TDN) at Corona Range and Livestock Research Center and were offered 20% CP supplement (Rancher Pro 20% Cube, Hi-Pro Feed, Friona, TX) 3d/wk for the duration of the study. Ruminal fluid was collected during April, June, August, and November of 2016 and February 2017. Samples were stored in -80^{o}C until subsequent analysis. Upon thawing ruminal fluid was analyzed for ammonia, VFA, and bacterial population composition.

Laboratory Analysis. Ruminal ammonia was analyzed using the phenol-hypochlorite procedure adapted to a microtiter plate (Broderick and Kang-Meznarich, 1980). Concentration of VFAs was determined by gas chromatography utilizing the methods of May and Galyean (1996). Total genomic DNA was extracted from samples utilizing a repeated beating plus column (RBB+C) method (Yu and Morrison, 2004) followed by 1 mL of lysis buffer using the QIAmp DNA Stool Mini Kit (Qiagen, Valencia, CA). Subsequent steps followed the extraction protocol directions in the kit. Quality and quantity of DNA was determined by a Nanodrop Spectrophotometer (Thermo Scientific, Marietta, OH).

Amplification and sequencing of the V4 variable region 16S rRNA gene was performed at by commercial laboratory (www.mrdnalab.com, Shallowater, TX, USA). Briefly samples were barcoded and PCR primers 515F/806R were used in a 28 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, with a final elongation step at 72°C for 5 minutes. A DNA library was prepared according to Illumina TruSeq DNA library preparation protocol. Sequencing was performed on a MiSeq (Illumina) following the manufacturer's guidelines. The PCR products were checked in a 2% agarose gel to determine application success and relative intensity of bands. Following PCR, all samples were pooled together in equal proportions based on molecular weight and DNA concentration. Samples were purified using calibrated Ampure XP beads. The DNA library was prepared by following Illumina TruSeq DNA library protocol, using purified PCR product. Sequencing was performed on a MiSeq following manufacturer's guidelines, and sequence data were processed using MR DNA analysis pipeline. Operational taxonomic units (OTUs) were generated from sequencing after removing < 150bp sequences. Final OTUs were clustered by 97% similarity and classified using BLASTn against a curated database derived from GreenGenes, RDPII and NCBI.

Statistical Analysis. All data were analyzed as a completely random design using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) with repeated measures for ruminal bacteria composition, VFA and NH_3. Animal was the experimental unit and treatment was the random variable. The model included the effect of day. Using Akaike's information criterion, compound symmetry was the covariance structure. Means were calculated using LSMEANS. Day effects were considered significant at a $P \leq 0.05$. When *F*-tests were significant, mean separations were performed using a pair-wise comparison (PDIFF).

RESULTS AND DISCUSSION

Dietary effects on rumen microbiome have long been understood (Hungate, 1966; Tajima et al., 2001). Sequencing of the V4 variable region 16S rRNA gene used in this study has further examined the effects of varying diet quality on the rumen microbiome. *Bacteroidetes* and *Firmicutes* were the predominant phylum throughout the study averaging 43.4% and 40.5% of the population, respectively. In most rumen microbiome studies *Firmicutes* and *Bacteroidetes* account for more than 90% of the 16S rRNA gene sequences in most of the reported data sets, but also at lower taxonomic levels (Kim et al., 2011; Creevey et al., 2014). When considering the effect of sampling month on phylum *Bacteroidetes* did not differ ($P = 0.82$) and *Firmicutes* was greater for June, August, and February ($P < 0.001$) compared to April and November. Common ruminal bacteria genera associated with the phylum *Firmicutes* include *Ruminococcus*, *Selenomonas*, and *Butyrivibrio.* Petri et al. (2013) investigated dietary effects on rumen microbiome composition using V3 hypervariable region of 16S rRNA gene. Rumen digesta was sampled from heifers fed forage, mixed concentrate

and forage, and high concentrate diets and found that *Bacteroidetes* and *Firmicutes* were the predominant phyla, but *Firmicutes* was greater comprising 10.4% more of the population than *Bacteroidetes* (Petri et al., 2013). Variations in results can be attributed to differing hypervariable regions and diet composition. The low protein and high structural carbohydrates found in native dormant forages is altered through weather and rain patterns, rapidly changing the forage quality and altering the rumen microbiome and fermentation parameters. Numerous data has examined the differences in concentrate and forage based diets (Tajima et al., 2001; Petri et al., 2013). Pitta et al. (2010) demonstrated the rumen microbiome adaptation to dietary changes when grazing bermudagrass hay or wheat grass and identified bacteria associated with different sample fractions (whole, liquid, and fiber). These data were the first to identify bacterial diversity associated with grass diets (Pitta et al., 2010). Shifts in the genera of bacteria in relation to season was also investigated in the present study. *Prevotella, Bacteroides*, *Clostridium, Butyrivibrio,* and *Succiniclasticum* were the predominant genera throughout the experiment. *Prevotella* and *Bacteroides* did not differ by sampling day ($P > 0.15$). *Prevotella* comprised approximately 17% of the population. These data conflicts with previously reported data from Petri et al. (2013) and Pitta et al. (2010). Petri et al. (2013) found *Prevotella* only comprised 8.9% of the population, while Pitta et al. (2010) described *Prevotella* and *Rikenella* as the prominent genera in all rumen content fractions. Kim et al. (2011) found *Prevotella* to be 11.1% of all bacterial sequences reviewed from the public databases. *Clostridium* differed by sampling day ($P < 0.01$) with the greatest population density occurring during February (7.7%). Differences in genera percentage can be attributed to analysis techniques, sampling methods and differing diets.

Shannon Weiner Index (**SWI**) and richness bacterial diversity indexes were evaluated and shown in Table 1. Richness refers to the number of different phyla or genera represented at one sampling. Richness did not differ at the phylum ($P = 0.15$) level but differences were observed at the genera ($P = 0.05$) level. The SWI is a quantitative measurement of the phylum and genera while considering the distribution of each individual phyla or genera. Shannon Weiner Index was calculated for phylum and genera and included all observed bacteria. Phylum SWI differed by sampling month with June and November ($P = 0.01$) having the greatest SWI compared to April, August, and November. Genera SWI was similar to phyla data with the exception that February ($P < 0.01$) was the greatest SWI differing from November, August, and April.

TABLE 1. Effect of advancing season on ruminal microbiome characteristics as assessed by richness and Shannon Weiner index

	2016				2017		
	Apr	Jun	Aug	Nov	Feb	SEM[1]	*P*-value
Phylum							
Richness	19.7	19.3	20.3	21.0	20.7	0.471	0.15
Shannon Weiner Index	1.0[a]	1.20[b,c]	1.17[b]	1.28[c]	1.15[b]	0.033	0.01
Genera							
Richness	215.0[a]	213.3[a,b]	204.3[b]	218.3[a]	220.7[a]	3.419	0.05
Shannon Weiner Index	1.9[a]	2.8[c]	2.8[c]	2.8[c]	3.5[b]	0.107	<.0001

[abc]values within rows with differing superscripts differ $P \leq 0.05$ between months.

[1]n=10.

These data differ from Pitta et al. (2010) but not Petri et al. (2013). Differences in SWI can be attributed to differing diets and diet qualities. More fibrous feedstuffs are colonized by closely adherent bacteria and often associated with secondary bacterial colonizers therefore, more fibrous feedstuffs result in greater colonization and diverse bacterial populations (Cheng et al., 1981; Flint and Bayer, 2008). The liquid fraction of rumen contents was collected in the present study. This methodology does not accurately represent the adherent population, reducing the adherent population representative, and thus explaining the differences in results seen from Pitta et al. (2010) and Petri et al. (2013).

Ruminal ammonia and VFA data are shown in Table 2. Ammonia levels are greatest in February and agree with total VFA production which was greatest in February ($P < 0.01$). Acetate concentrations ($P < 0.01$) decreased throughout the year, while propionate ($P < 0.05$) increased resulting in the highest acetate:propionate ($P < 0.01$) in February. Isobutyrate concentrations did not change ($P > 0.05$) and butyrate concentrations increased from April to August however, February had the greatest butyrate concentration ($P < 0.01$). Ruminal ammonia values differed by collection month ($P < 0.01$). Total VFA concentrations were less than previously reported data from Choat et al. (2003), but comparable acetate, propionate, isobutyrate, and butyrate were observed (Choat et al., 2003). Volatile fatty acids represent the main supply of metabolizable energy for ruminants (Van Soest, 1982), a reduction in total VFA production would be energetically unfavorable for the nutrition of the animal (Busquet et al., 2006). Animal performance is enhanced through nutrient synchrony. During our study VFA concentrations increased in sync with increasing diet quality. Microbial carbohydrate metabolism is to release ATP for microbial growth (Hoover and Stokes,

TABLE 2. Effect of advancing season ruminal ammonia and VFA concentrations over a one year sampling period.

	2016				2017		
	Apr	Jun	Aug	Nov	Feb	SEM[1]	*P*-value
Total VFA, mM	45.3[a]	56.8[a]	88.0[b]	87.1[b]	117.1[c]	8.769	< 0.01
VFA, mol/100 mol							
Acetate	71.7[a]	73.3[a]	72.5[a]	70.9[a]	66.2[b]	0.942	< 0.01
Propionate	16.7[ac]	14.1[b]	18.8[ab]	16.9[ac]	18.6[c]	0.835	< 0.05
Isobutyrate	2.0[a]	1.8[ab]	1.5[b]	1.6[ab]	1.3[b]	0.173	0.82
Butyrate	8.0[a]	8.6[ab]	9.5[b]	8.8[ab]	12.7[c]	0.415	< 0.01
Acetate: Propionate	4.3[ac]	5.2[b]	4.9[ab]	4.2[ac]	3.6[c]	0.265	< 0.01
Ammonia, mM	4.1[a]	2.8[a]	6.7[ab]	8.0[b]	17.0[c]	1.422	< 0.01
pH	7.1[a]	7.2[a]	6.6[ab]	6.9[a]	6.1[b]	0.20	0.03

[abc]values within rows with differing superscripts differ $P \leq 0.05$ between months.

[1]n=10.

1991). Microbial nitrogen metabolism rates are dependent on rate of carbohydrate metabolism (Hoover and Stokes, 1991). Satter and Slyter (1974) reported maximum microbial growth at 2 mM ruminal ammonia. These data demonstrates that despite native range with low protein and high structural carbohydrates, rumen function was not impaired during the duration of this research. February appears to be the most optimal month with the greater potential for nutrient synchrony, in terms of bacterial diversity and rumen function. Ruminal pH was in agreement with total VFA and ammonia data. Ruminal pH was more acidic for February which also had the greatest total VFA production.

IMPLICATIONS

Results show the rumen microbiome and fermentation parameters fluctuate with varying diet quality over a year on native dormant rangeland. Core microbial populations remain major components of the rumen bacterial population however, variations in diet quality can result in differing ratios of the microbial population. Ruminal ammonia and VFA concentrations will fluctuate in favor of diet quality. As the seasons and rainfall patterns change the rumen microbial population and fermentation parameters reflect those changes. These changes observed highlight an important supplementation issue, that diet quality fluctuates throughout the year and supplementation programs should be adjusted as such to ensure nutrient synchrony and rumen efficiency. Further research should be conducted to examine adaptation time of ruminants to varying quality on dormant native rangeland.

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In utero inhibition of chemokine receptor four signaling alters peripheral blood immune response during early pregnancy in ewes[1]

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ABSTRACT: Fetal tolerance integral to pregnancy maintenance extends to altered immune cells in maternal circulation, and chemokine signaling may be central to regulating immune cell functions. We previously reported a reduced percentage of phenotypically pro-inflammatory (CD8) immune cells expressing chemokine receptor 4 (CXCR4) in systemic circulation when CXCR4 signaling was inhibited at the fetal-maternal interface in ewes during early gestation. These results concluded that CXCR4 signaling in the uterus affects peripheral blood immune cell phenotype, and may also modulate systemic inflammatory cytokine balance. With this information, our hypothesis was that antagonizing CXCR4 in utero would result in gene expression indicative of an anti-inflammatory environment in peripheral blood, spleen, and corpus luteum (CL). To test this hypothesis, we surgically installed osmotic pumps to infuse a CXCR4 inhibitor (AMD3100) or PBS to the fetal-maternal interface in ewes beginning on d 12 of gestation. Spleen and CL tissues were collected on d 20 and 35, and blood was collected daily starting on d 10 of gestation. Gene expression of inflammatory cytokines and chemokine ligand 12 (CXCL12) and its two receptors was investigated using quantitative PCR. In circulating blood of AMD3100-treated ewes, transcript for transforming growth factor beta 1 (TGFB1) increased on d 14 ($P < 0.01$) and 16 ($P < 0.05$), while *IL10* declined on d 26 ($P < 0.05$) and had tendency to be less on d 28 ($P = 0.09$) compared to control. In spleen tissue, *TGFB1* was elevated ($P < 0.05$) on d 20 in treated ewes, while *IL10* declined ($P < 0.05$) on d 35 compared to control ewes. Corpora lutea had less interferon gamma (*IFNG*) ($P < 0.05$) and greater *CXCR7* ($P < 0.05$) in treated ewes on d 35 of gestation. These results in part confirm our hypothesis and provide further insight to CXCL12-CXCR4-CXCR7 regulation of inflammation during pregnancy and function of its signaling in fetal-maternal tolerance.

Key words: chemokine receptor 4, corpus luteum, cytokine, early gestation, immunology, spleen
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INTRODUCTION

With a 50% projected increase in world food demand by 2030 (OECD, 2010), a better understanding of basic mechanisms contributing to pregnancy success is needed to enhance reproductive efficiency. Leukocytes can dictate acceptance of the conceptus not only in the uterus, but also in circulating blood (Maeda et al., 2013; Lee et al., 2015). Peripheral cytotoxic T (**Tc**) cell numbers do not change during bovine pregnancy, but alternately reduce synthesis of pro-inflammatory cytokine tumor necrosis factor (**TNF**) compared to non-pregnant females (Oliveira and Hansen, 2008). Leukocyte and cytokine population dynamics in reproductive tissues are partially regulated by chemokines (Baggiolini, 1998). Activation of chemokine ligand 12 (**CXCL12**) signaling through chemokine receptor 4 (**CXCR4**) aids trophoblast cell survival, implantation, and immune cell migration (Jaleel et al., 2004; Ren et al., 2012; Quinn et al., 2014). Indeed, inhibiting CXCR4 signaling with its antagonist AMD3100 at the fetal-maternal interface alters inflammatory cytokine expression in caruncle and fetal extraembryonic membranes (Prosser et al., 2016; Quinn et al., 2017). Fetal-maternal tolerance extends to immune modulation in circulation, possibly in part due to chemokine signaling. In utero treatment with AMD3100 results in declined numbers of circulating $CXCR4^+$ Tc cells

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during early gestation (Prosser et al., 2016). To further elucidate implications of the modified systemic leukocyte phenotype provoked by antagonizing CXCR4 in utero, this study examines the expression profile of systemic inflammatory cytokines under similar conditions. We hypothesized that in utero inhibition of CXCL12-CXCR4 signaling would encourage gene expression signifying an anti-inflammatory environment in peripheral blood, spleen, and corpus luteum (**CL**).

MATERIALS AND METHODS

Animals and Tissue Collection

Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Rambouillet-cross ewes received intravaginal controlled internal drug release (CIDR) inserts for 5 d to synchronize estrus and two injections of dinoprost tromethamine (5 mg i.m.; Zoetis, Parsippany-Troy Hills, NJ) administered 4 h apart following CIDR removal. The study used a total of 27 animals. Ewes were mated by a fertile ram and randomly placed into experimental groups of either control (**CON**) or treatment (**AMD3100**). Osmotic pumps (Alzet, Cupertino, CA) were filled with AMD3100 (2060 ng; Selleckchem, Houston, TX) or PBS (CON) according to manufacturer's instructions. On d 12 of gestation, ewes were anesthetized (5 mg xylazine and 100 mg ketamine; 1 mL i.v.) and maintained on isofluorane. A catheter attached to the pump was introduced into the uterine lumen ipsilateral to CL, emptying treatment into the uterine lumen. Blood samples were collected daily from d 10 through d 35 of pregnancy via jugular venipuncture into 10 mL EDTA vacutainer tubes (VWR, Radnor, PA), which were subsequently centrifuged at 4°C for 30 min at 3,750 × *g* and buffy coats were collected for RNA extraction. On d 20 or d 35 of gestation, ewes were anesthetized with sodium pentobarbital (20 mg/kg i.v.) and reproductive tracts were removed via mid-ventral laparotomy. Spleen and CL tissue were collected with sterile technique, snap frozen in liquid nitrogen, and stored at -80°C until RNA isolation. Ewes were euthanized by exsanguination while under anesthesia.

RNA Isolation and Quantitative PCR (qPCR)

Total RNA was extracted per manufacturer's instructions using 750 µL of Tri-Reagent BD (Molecular Research Center Inc., Cincinnati, OH) supplemented with 30 µL of 5 *N* acetic acid for buffy coats and 1 mL of Tri Reagent (Molecular Research Center Inc., Cincinnati, OH) per 100 mg of tissue for CL and spleen. All RNA samples were eluted with nuclease-free water. Ribonucleic acid from buffy coats was treated with RNase-free DNase (Qiagen, Hilden, Germany) prior to purification with the RNease MinElute Cleanup Kit (Qiagen, Hilden, Germany), while RNA from CL and spleen was treated with DNase using the TURBO DNA-free kit (Ambion, Foster City, CA). Quantity and purity of RNA were determined using a NanoDrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA). Ribonucleic acid samples were stored at -80° for further analysis.

TABLE 1. Primer sequences for each ovine gene of interest

Gene	Reverse primer sequence	Forward primer sequence
GAPDH	5'-CGTTCTCTGCC TTGACTGTG-3'	5'-TGACCCCTTCA TTGACCTTC-3'
CXCL12	5'-GGTCAATGCAC ACTTGCCTA-3'	5'-CCTTGCCGAT TCTTTGAGAG-3'
CXCR4	5'-ATTTTCCTCCC GGAAGCAGG-3'	5'-GGGATCCGTAT ATTCACTTCCGA-3'
CXCR7	5'-AAGTAGGCGA CGGACAGGTA-3'	5'-CCATCAACCTC TTTGGCAGC-3'
IFNG	5'-TCTCCGGCCT CGAAAGAGAT-3'	5'-GGCTGATTCAA ATTCCGGTGG-3'
TNF	5'-TCAGGTAAAG CCCGTCAGTG-3'	5'-GTAGCCCACGT TGTAGCCAA-3'
IL12A	5'-TCCAGAAGACA GACAATGCCC-3'	5'AGCCACGAAT GAGAGTTGCC-3'
IL10	5'-ACACCCCTC TCTTGGAGCAT-3'	5'-GGCGCTGTCA TCGTTTTCTG-3'
TFGB1	5'-CCGGAACTGA ACCCGTTGAT-3'	5'-AGAAGGCTTTC GCCTCAGTG-3'

Synthesis of cDNA and qPCR analysis were completed as previously described (Quinn et al., 2014) using primers listed in Table 1. Relative gene expression was graphed with 2-ΔΔCq.

Statistical Analysis

Target gene Cq values were normalized to that of glyceraldehyde phosphate dehydrogenase for each sample using the ΔCq method (Schmittgen and Livak, 2008). Data were analyzed using $2^{-\Delta\Delta Cq}$ values, and significant changes were determined at $P < 0.05$ with an unpaired, two-tailed Student's *t*-test using Prism (Version 6; GraphPad Software Inc., La Jolla, CA).

RESULTS AND DISCUSSION

We previously reported a decline in peripheral blood $CXCR4^+$ Tc cells following AMD3100 treatment at the fetal-maternal interface in sheep (Prosser et al., 2016) implying a mechanism by which CXCR4 signaling governs peripheral immune response during early pregnancy. To determine consequences of this

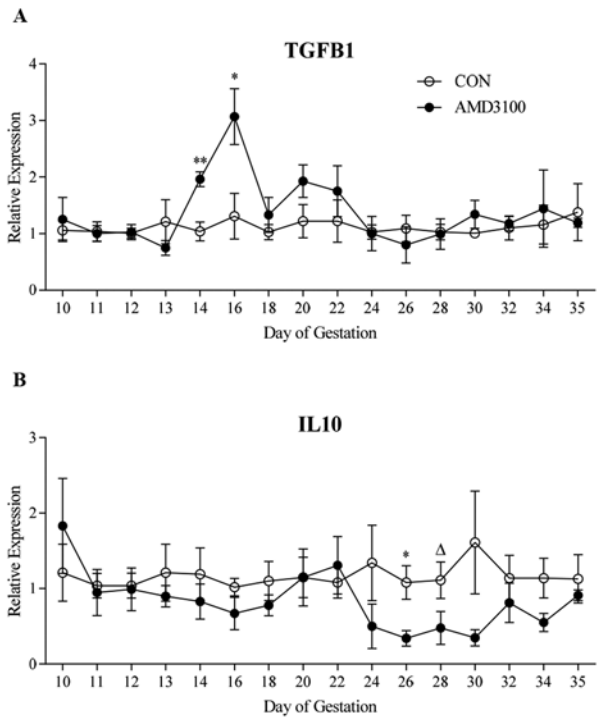

Figure 1. Peripheral blood anti-inflammatory cytokine mRNA expression was differentially affected by CXCR4 inhibition at the fetal-maternal interface. AMD3100 treatment was administered on d 12 of pregnancy. Expression of transforming growth factor beta 1 (TGFB1) rose in treated (AMD3100) compared to control (CON) on d 14 and 16 ($P < 0.01$ and $P < 0.05$, respectively; A). Interleukin 10 declined on d 26 ($P < 0.05$) and had tendency for reduction on d 28 ($P = 0.09$; B) in AMD3100. Data are represented by graphing $2^{-\Delta\Delta Cq}$. Graphs represent the mean ± SEM with significant differences between CON and AMD3100 denoted by asterisk.

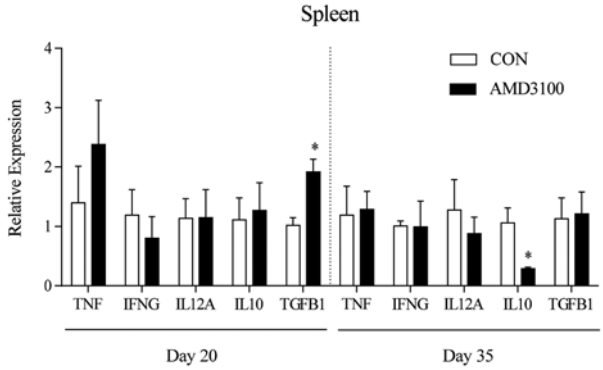

Figure 2. Differential effects of CXCR4 inhibition at the fetal-maternal interface on transforming growth factor beta 1 (TGFB1) and IL10 mRNA expression in spleen. AMD3100 treatment was administered on d 12 of pregnancy. On d 20 of gestation, *TGFB1* rose ($P < 0.05$) in treated ewes (AMD3100) compared to control (CON), while *IL10* was less ($P < 0.05$) in AMD3100 on d 35 of pregnancy. Data are represented by graphing $2^{-\Delta\Delta Cq}$. Graphs represent the mean ± SEM with significant differences between CON and AMD3100 denoted by asterisk.

phenotypic alteration, pro-inflammatory (TNF; interferon gamma, **IFNG**; IL12A) and anti-inflammatory (transforming growth factor beta 1, **TGFB1**; IL10) cytokines were evaluated in circulating immune cells, spleen, and CL. Notably, *TGFB1* was elevated in circulating white blood cells on d 14 ($P < 0.01$) and d 16 ($P < 0.05$) from ewes treated with AMD3100 in utero compared to CON (Fig. 1). Expression of *IL10* in AMD3100 treated ewes was reduced on d 26 ($P < 0.05$) in circulating white blood cells and tended to decline on d 28 ($P = 0.09$) compared to CON (Fig. 1). All other targets were detected but did not differ.

Cytotoxic T cells are pro-inflammatory mediators of antiviral immunity, capable of recognizing pathogenic cells that express an antigen of interest and injecting peptidases in those target cells to induce programmed cell death. Our hypothesis was that cytokine gene expression would signify an anti-inflammatory environment in tissues examined. Elevation of *TGFB1* expression in peripheral blood, although temporal, was consistent with our hypothesis and may be result of the decline in Tc lymphocytes reported on d 15 (Prosser et al., 2016). Declined *IL10* is curious and deserves further exploration to discern its significance.

The spleen is a dedicated and dynamic organ responsible not only for recycling erythrocytes, but also clearance of blood-borne pathogens. Splenic antigen-presenting cells are capable of priming Tc lymphocytes, therefore changes observed in circulation were likely to manifest also in spleen. When tissue was harvested on d 20 and 35, *TGFB1* was elevated ($P < 0.05$) on d 20 and *IL10* was decreased ($P < 0.05$) in spleen on d 35 in AMD3100 compared to CON ewes (Fig. 2). Similar to periphery, all other targets were detected in tissue from both collections, but did not change with treatment. Remarkably, *TGFB1* and *IL10* were both modified in spleen 4 to 8 d following changes in expression in circulation. Whether this is demonstrative of delayed splenic immune response or lasting consequential cytokine production still needs to be understood. Additionally, aberrations in inflammatory cytokine expression have consequences on intermediary metabolism (reviewed by Gifford et al., 2012). Because these Tc lymphocytes are memory T cells, effects on offspring and maternal growth performance due to altered immune cell phenotype deserve exploration.

Oakley et al. (2010) described tissue-specific splenocyte mobilization to the ovaries promoting ovulation; if leukocytes are alternately activated in the spleen and travel to the ovary with a different phenotype, this may also have consequences on the CL. As the main source of progesterone during early pregnancy, it was important to evaluate the CL for shifts in inflammatory cytokine expression. On d 35 of gestation, expression of pro-inflammatory *IFNG* declined ($P < 0.05$) in AMD3100 ewes compared to CON while all other cytokines were unaffected ($P > 0.10$; Fig. 3).

A decline in IFNG may be beneficial to CL function, as treatment of luteinized granulosa cells in vitro with IFNG inhibits progesterone production in a

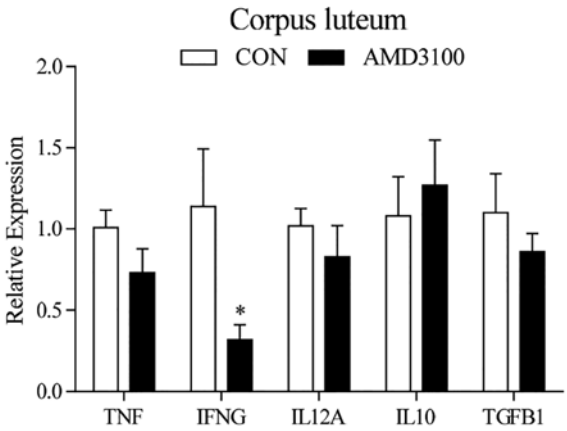

Figure 3. Expression of interferon gamma (IFNG) mRNA declines in corpus luteum (CL) following AMD3100 treatment at the fetal-maternal interface. AMD3100 treatment was administered on d 12 of pregnancy. On d 35 of gestation, *IFNG* was less ($P < 0.05$) in CL of treated ewes (AMD3100) compared to control (CON). Data are represented by graphing $2^{-\Delta\Delta Cq}$. Graphs represent the mean ± SEM with significant differences between CON and AMD3100 denoted by asterisk.

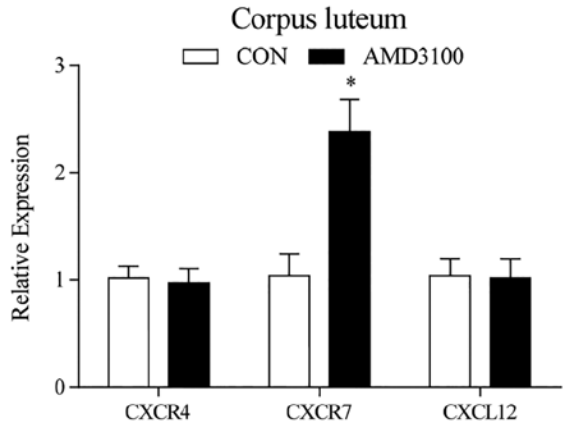

Figure 4. Chemokine receptor 7 (CXCR7) mRNA expression elevates in corpus luteum (CL) following AMD3100 treatment at the fetal-maternal interface. AMD3100 treatment was administered on d 12 of pregnancy. On d 35 of gestation, *CXCR7* was greater ($P < 0.05$) in CL of treated ewes (AMD3100) compared to control (CON). Data are represented by graphing $2^{-\Delta\Delta Cq}$. Graphs represent the mean ± SEM with significant differences between CON and AMD3100 denoted by asterisk.

dose-dependent manner and PG synthesis in bovine luteal cells is stimulated following IFNG treatment (Best et al., 1995; Pate, 1995). However, growing evidence suggests that pro-inflammatory cytokines play a supportive role in early CL growth in rats and horses (Brannstrom et al., 1993; Galvao et al., 2013) and IFNG-secreting cells are higher in peripheral blood of pregnant women than non-gravid women (Matthiesen et al., 1998). Indeed, *IFNG* expression is elevated in ovine CL on d 25 of gestation compared to d 10 of the estrous cycle (Prosser et al., 2016), indicating a sustaining role of this pro-inflammatory cytokine. In the present study, no change was observed in cytokine expression on d 20 ($P > 0.10$; data not shown); therefore, CL maintenance appears to involve tightly controlled presence of pro-inflammatory cytokines.

The CXCL12-CXCR4 chemokine-receptor pair initiates multitudinous signaling cascades with outcomes including stimulation of monocyte differentiation into anti-inflammatory macrophages, T cell migration, and placental development (Sanchez-Martin et al., 2011; Werner et al., 2011). Chemokine receptor 7 was recently found to bind CXCL12, but thought to solely sequester CXCL12, thereby preventing it from binding CXCR4 (Singh et al., 2013). More recently reports of CXCL12 signaling through CXCR7 have arisen, demonstrating similar biological events to CXCL12-CXCR4 such as cell survival and regulation of vascular endothelial growth factor (Wang et al., 2008; Tripathi et al., 2014). Thus, observing expression of *CXCL12* and its receptors in CL may give insight to the noted changes in *IFNG*. While *CXCL12* and *CXCR4* remained stable ($P > 0.10$; Fig. 4), expression of its other receptor, *CXCR7*, was greater in CL from d 35 AMD3100 ewes compared to CON ($P < 0.05$; Fig. 4). Although *CXCR7* increased concurrently with *IFNG* decline in CL of d 35 AMD3100 ewes, production of CXCR7 by leukocytes remains inconclusive (reviewed by Singh et al., 2013). A follow-up study will help establish the impact of *CXCR7* expression changes in AMD3100-treated ewe CL, but at this time it is not possible to confirm CXCR7 involvement in the declined *IFNG* in CL of treated ewes.

Nevertheless, elevated *CXCR7* expression in CL does have potentially striking implications. Production of CXCR7 increases when CXCR4 synthesis is knocked down in prostate cancer cells (Wang et al., 2008), but to our knowledge this has not been demonstrated in vivo using a CXCR4 antagonist. Decidedly different mechanisms of inhibition and knockdown of CXCR4 lead us to speculate that lack of CXCR4 downstream signaling may stimulate CXCR7 transcription as compensation. However, why a mirrored change did not occur on d 20 or in other tissues must also be explained.

Through inhibiting CXCR4 signaling at the fetal-maternal interface in sheep and exploring inflammatory cytokine gene expression in peripheral tissues, this study emphasizes the importance of CXCL12-CXCR4-CXCR7 signaling during early pregnancy, and significance this axis may have not only in the uterus for fetal-maternal tolerance, but in the whole body and potentially on offspring performance.

IMPLICATIONS

Systemic inflammatory cytokine expression was impacted by inhibiting chemokine receptor 4 signaling in the uterus of ewes during early gestation in this study. Additional research is needed to clarify whether this is through leukocyte modification at the fetal-maternal interface or by another mechanism. These data suggest that chemokine signaling affects homeostasis and fetal-maternal tolerance not only in the uterus, but the entire animal. This valuable information will contribute to ameliorating embryonic loss in the livestock industry through therapeutic developments. Because CD8 cells are memory T cells, further exploration on offspring growth is deserved.

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Influence of increased nutrient intake before and after breeding on performance and reproductive efficiency of beef heifers

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Abstract: One hundred three spring-born Angus crossbred heifers were utilized over a 2 yr period to determine the effect of pre- and post-breeding nutrition on heifer performance and reproductive efficiency. Heifers were developed grazing dormant forage and allotted randomly to 1 of 2 treatments 1) a constant gain of 0.5 kg/d for the entire development period (CG) or 2) to gain 0.25 kg/d for approximately the first 52 d, followed by 0.75 kg/d for the remaining 38 d (LoHi). Pre-breeding supplementation was initiated in February and terminated at the onset of breeding season (mid-May). Heifers were then subdivided and assigned to 1 of 2 post-breeding treatments 1) targeted gain of 0.4 kg/d (CON) or 2) targeted to gain 0.75 kg/d (High) for 30 d to determine the effect of post-breeding ADG on conception rates. Post-breeding heifers grazed dormant forage and were supplemented 30 d following breeding. Heifers were subjected to ovarian ultrasonography to measure antral follicle counts and reproductive tract score (RTS). Heifers were synchronized utilizing the 7-d CIDR-PG protocol with AI following estrus detection. Heifer initial, mid, and final pre-breeding BW was not different ($P \geq 0.31$). Average daily gain was similar between treatments for period 1 and 2 ($P \geq 0.48$) with no difference ($P = 0.20$) in total ADG for the pre-breeding development period. Antral follicle count, RTS, and proportion of heifers attaining puberty prior to the breeding season were similar among treatments ($P \geq 0.53$). Post-breeding treatment did not affect ($P \geq 0.25$) BW or ADG. Estrus response was similar ($P = 0.67$) among treatments, however, there was a trend ($P = 0.10$) for CON heifers to have an increased AI pregnancy rate. Nonetheless, final pregnancy rates did not differ ($P = 0.72$). Accurately predicting forage intake and quality impacted performance, altering predicted gains. In yr 2, 8 ruminally cannulated heifers were utilized to quantify differences in forage nutritive quality. Masticate samples were up to 8 percentage points greater in CP compared to clipped samples indicating greater forage selectivity for grazing heifers. Based on these data, rate of supplementation did not impact growth parameters or reproductive efficiency of beef heifers, due in part to diet selectivity of heifers grazing native rangelands.

Key words: heifer development, nutrition, reproduction
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INTRODUCTION

Recently heifer development strategies have focused on increasing economic efficiency through reducing inputs. These systems limit nutrient intake and take advantage of compensatory gains, through nutrient restriction early in the development period to improve AI pregnancy rates (Lynch et al., 1997; Summers et al., 2014). Research investigating the impact of improved nutrient intake prior to the breeding season has been limited, reporting only phenotypic results, failing to examine mechanisms behind improved reproductive success.

Post-insemination diet and management influence AI pregnancy rates and embryonic survival (Perry, 2012). Heifers managed in a drylot during the development period and placed in a forage grazing situation had decreased AI pregnancy rates compared with contemporaries developed in a grazing situation (Perry et al., 2013). Additionally, nutrient restriction immediately following AI resulted in impaired development of d-6 embryos (Bridges et

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al. 2012). Elucidating the impact of nutrition on reproductive function may allow for an increase in the proportion of heifers successfully becoming pregnant early in the breeding season, increasing productivity.

Based on these data we hypothesized that increasing heifer nutrient intake prior to the breeding season and during early gestation would improve heifer conception rates. Therefore, the objective of this study was to determine the effects of increased nutrient intake pre- and post-breeding on heifer performance and reproductive efficiency.

MATERIALS AND METHODS

All animal procedures and facilities were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Pre-breeding heifer management

Over 2 yr, 103 spring-born British crossbred heifers (238 kg BW at weaning) were used to compare 2 supplementation strategies for developing heifers on native dormant range at the New Mexico State University Corona Range and Livestock Research Center (CRLRC) located 13 km east of Corona, NM (34°15′36″ N, 105°24′36″ W).

Heifers were weaned in early October each yr, at which time heifer BW was recorded. Following weaning, all heifers were managed together and grazed a common pasture until the initiation of treatments. Treatments were initiated mid-February each yr with heifers stratified by BW, breed, and randomly assigned to 1 of 2 pre-breeding nutritional regimes: constant gain (CG) with heifers targeted to gain 0.4 kg/d or low-high (LoHi) targeted to gain 0.25 kg/d for approximately the first 52 d of the development period followed by 0.75 kg/d for the remaining 38 d of the development period. Heifers were targeted to achieve 55% mature BW prior to the breeding season. Treatments were assigned to 2 of 4 pastures, resulting in 2 replications per treatment within each of the 2 yr.

In yr 1 forage clippings were collected in March, May, and June utilizing a 0.25-m^2 quadrat. Upon collection forage samples were analyzed for CP and NDF (SDK Laboratories, Inc., Hutchinson, KS). Forage samples averaged 5.1, 6.0, and 6.0% CP and 70.3, 71.5, and 69.9% NDF for March, May, and June respectively. In yr 2 cannulated heifers (n = 8) were used to collect diet extrusa samples for analysis of CP, TDN, and NDF (SDK Laboratories, Inc.). Rumen cannulated heifers grazed alongside herd mates to obtain diet extrusa samples in each of the 4 pastures. Extrusa samples were collected monthly throughout the pre- and post-breeding treatment periods via the ruminal evacuation techniques described by Lesperance et al. (1960). Ruminal extrusa samples were gently rinsed with 500 mL of deionized water, dried in a forced air oven at 55°C and mixed thoroughly every 12 h until completely dried and ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 2 mm screen. Forage clippings were collected concurrently with extrusa samples utilizing a 0.25-m^2 quadrat to ensure forage availability did not limit heifer productivity (965 kg/ha ± 406 kg) and analyzed for CP, NDF, and TDN (SDK Laboratories Inc.).

Heifers were offered a 20% CP supplement (Rancher Pro 20% Cube, Hi-Pro Feed, Friona, TX; 23.4% CP, 44.8% TDN, DM basis) 3 times weekly. Constant gain heifers were supplemented at a rate of approximately 2.39 kg·heifer^{-1}·d^{-1} over the entire 90 d development period, LoHi heifers were supplemented at a rate of approximately 0.88 kg·heifer^{-1}·d^{-1} for approximately the first 52 d and 2.88 kg·heifer^{-1}·d^{-1} for approximately the last 38 d of the treatment period. Supplementation period was based on targeted performance each yr. Heifers had unlimited access to water and a loose salt-mineral mix formulated to complement available forage. The loose-salt mineral was composed of 10% Ca, 7% P, 2% Mg, 0.5% K, 2,500 ppm Cu, 5,000 ppm Zn, 2,500 ppm Mn, 75 ppm I, 15 ppm Se, and 120,000 units/lb vitamin A (Hi-Pro Feed). Heifer BW and BCS were recorded biweekly over the entire treatment period to monitor gains.

Estrus was synchronized utilizing the 7-d CIDR-PG protocol (CIDR; Eazi-Breed, Zoetis Animal Health, Florham Park, NJ). Heifers received a CIDR insert for 7 d after which the CIDR was removed and all heifers received a single 5-mL i.m. injection of $PGF_{2\alpha}$ (Lutalyse, Zoetis Animal Health). At the time of CIDR removal an estrus detection aid (Estrotect, MAI Animal Health, Elmwood, WI) was applied. Heifer development treatments were terminated at the onset of the breeding season and heifers were placed in a common pasture and estrus detection performed for 5 d following $PGF_{2\alpha}$ administration. Heifers were AI approximately 12 h after observed standing estrus. Approximately 10 d following the last day of AI, heifers were exposed to bulls at a ratio of 1 bull to 14 heifers for approximately 45 d. Artificial insemination rates were determined 30 d after AI via blood sample. Overall pregnancy rates were determined approximately 44 d after bull removal via transrectal ultrasonography.

Post-breeding Heifer Management

Following breeding heifers were stratified by pre-breeding treatment, BW, estrus response, and randomly assigned to 1 of 2 post-breeding supplementation treatments: control (Con) receiving the equivalent of 0.45 kg·heifer^{-1}·d^{-1} or high (High) receiving the equivalent of 2.27 kg·heifer^{-1}·d^{-1} of a 20% CP supplement (Rancher Pro 20% Cube, Hi-Pro Feed) 3 times weekly over 30 d treatment period. Treatments were assigned to 2 of 4 pastures, resulting in 2 replications per treatment within each of the 2 yr. Heifers had unlimited access to water and a loose salt-mineral mix formulated to complement available forage. Heifer BW and BCS were recorded weekly over the entire treatment period to monitor gains. Following termination of post-breeding treatment, heifers were managed together through the calving season.

Blood Collection and RIA

A single blood sample was collected biweekly (pre-breeding) or weekly (post-breeding). Samples were collected via coccygeal venipuncture into serum separator vacuum tubes (Corvac, Kendall Healthcare, St. Louis, MO). Samples were allowed to clot at room temperature for approximately 30 min then subjected to centrifugation (1,200 × *g* for 20 min at 4ºC). Serum was harvested and stored at -20ºC until assayed. Serum progesterone (P4) concentrations were quantified by RIA using components of a solid phase kit (MP Biomedicals, LLC, Santa Ana, CA) and modified for use in ruminant serum as reported by Schneider and Hallford (1966). Intra- and inter-assay coefficients of variation were 11.5 and 7.4%, respectively. Heifers with serum P4 >1.0 ng/mL were considered pubertal (Henricks et al., 1971).

Statistical Analysis

Data were analyzed utilizing the MIXED and GLIMMIX procedures of SAS (SAS Inst. Inc., Cary, NC). Heifers were developed utilizing 4 pastures each year with 2 pastures per treatment, and treatments were repeated for 2 yr. Pasture was considered the experimental unit. The initial model for the pre-breeding period included pre-breeding heifer development treatment and the interaction of heifer development treatment × year. The interaction was not significant and was removed from the model. The initial model for the post-breeding period included post-breeding heifer supplementation treatment and the interaction of post-breeding supplementation treatment × pre-breeding heifer development treatment. The interaction was not significant and was removed from the model. A P-value ≤ 0.05 was considered significant.

TABLE 1. Effect of heifer pre-breeding development system on heifer BW, ADG, and percent mature BW

Item	CG[1]	LoHi[2]	SEM	*P*-value
n	4	4		
Pre-breeding BW, kg				
Initial	232	231	4	0.84
Mid	250	243	4	0.31
Final	276	271	4	0.45
ADG, kg/d				
Period 1[3]	0.34	0.22	0.11	0.48
Period 2[4]	0.59	0.61	0.05	0.74
Overall[5]	0.48	0.42	0.03	0.20
Mature BW, % [6]	54	53	0.87	0.50
AFC[7]	22.6	21.8	1.12	0.92
RTS[8]	4.3	4.5	0.11	0.53
Pubertal, %	88	88	8.43	0.97
Estrus response, %	74	78	6.75	0.67

[1]CG= heifers were supplemented to gain approximately 0.4 kg/d over the 90 d development period while grazing native range.

[2]LoHi = heifers were supplemented to gain approximately 0.25 kg/d for the first portion of the development period followed by 0.75 kg/d for the remaining portion of the development period while grazing native range.

[3]Period 1= the first approximately 52 d.

[4]Period2= the remaining approximately 38 d.

[5]Overall= the entire 90 d development period.

[6]Based on herd average mature cow BW (512 kg).

[7]Antral follicle count.

[8]Reproductive tract score.

RESULTS AND DISCUSSION

Pre-breeding heifer performance

Heifer BW and ADG for the pre-breeding development period are reported in Table 1. Heifer initial, mid, and final BW were similar ($P \geq 0.31$) among treatments. Furthermore, percent mature BW achieved prior to the breeding season did not differ ($P = 0.50$) between CG and LoHi heifers, with heifers achieving 53.5% mature BW. Average daily gain did not differ ($P \geq 0.20$) among treatments at any time point. Lynch et al. (1997) reported heifers fed to achieve an even gain over the entire treatment period or a delayed gain, had similar reproductive performance, proving BW gains could be delayed until late in the development period without compromising reproductive success.

The current study was based on previous research reported by Summers et al. (2014) to further investigate improved AI pregnancy rates in heifers developed in a reduced input system. In that study, heifers were developed either in a drylot (DL) or grazing corn

residue (CR) and winter range during the development period and offered the equivalent of 0.45 kg/d of 32% CP supplement. Heifers developed grazing CR were placed in the drylot with DL heifers for 40 d prior to the breeding season for estrous synchronization, during which time ADG tended to be greater for CR heifers. Corn residue developed heifers had an 11 percentage point increase in AI pregnancy rates compared to contemporaries developed in the drylot, although developed to a reduced proportion of mature BW. Increases in AI pregnancy rate could be attributed to increased nutrient intake and BW gain by CR heifers during the period immediately before breeding, potentially resulting in a compensatory gain effect (Summers et al., 2014). Development of the preovulatory follicle from the early antral stage to ovulation takes approximately 40 d (Wathes et al., 2007). This crucial period of development coincides with increased BW gains, and suggests the compensatory gain experienced by CR heifers potentially caused a nutritionally induced flushing effect, positively impacting AI pregnancy rates through enhanced oocyte competence. Current research sought to expand upon these results, investigating if similar results were possible developing heifers grazing native range. However, developing heifers grazing native forage created several challenges.

In yr 1 supplementation rates were calculated based on historic forage values and adjusted throughout the development period to achieve predicted ADG. However, an inability to accurately predict forage intake in a range setting effected gains throughout the experiment. Adjusting supplement rates in yr 1 was not successful in achieving the desired performance, thus in yr 2, supplementation rates were not adjusted, rather were based on yr 1 forage values. In yr 2 to determine the nutrient composition of the native forage consumed by heifers, 8 heifers were ruminally cannulated and grazed alongside contemporaries. Masticate samples and forage clip samples were collected monthly (data not shown).

Characterizing the nutritional value of available forage becomes difficult due to diet selectivity of the animal while grazing. Typically, grazing cattle consume a higher quality diet compared to clipped forage samples, and an estimated 2% increase in protein has been suggested as a standard when comparing the nutritive value of clipped and esophageal samples (Simms, 2013). When comparing the clipped and masticate samples collected in yr 2 a slight increase was reported between samples taken on March 1 and 31, with a 1.6% and 1.7% increase in CP for masticate samples, respectively (Rosasco, 2016). However, when comparing clipped and masticate samples taken on April 26, May 23, and June 22 an increase ($P \leq 0.01$) in CP (6.4%, 8.5%, and 5.4% increase, respectively) is reported for masticate samples (Rosasco, 2016). The increase in CP of masticate samples can be associated with a rise in ambient temperatures, increasing soil temperatures, allowing plant growth to begin. Heifers were able to select a higher quality diet of vegetative forage versus dormant forage. Based on clipped samples heifers were grazing a low-quality forage with an average CP of 5.4% and supplementation was formulated to meet protein requirements and achieve desired BW gains. However, heifers consistently selected a considerably higher quality diet that would have altered supplementation rates.

TABLE 2. Effect of heifer post-breeding supplementation on heifer BW, ADG, and reproductive performance

Item	CON[1]	High[2]	SEM	*P*-value
n	4	4		
BW, kg				
Initial	264	271	5	0.33
Mid	286	294	5	0.34
Final	291	294	5	0.75
Total ADG,[3] kg/d	0.85	0.69	0.09	0.25
AI pregnancy rate, %	86	64	8	0.10
Final pregnancy rate, %	95	93	6	0.72

[1]Con=heifers were supplemented at a rate of 0.45 kg·heifer^{-1}·d^{-1} 3 times weekly while grazing native range.

[2]High=heifers were supplemented at a rate of 2.27 kg·heifer^{-1}·d^{-1} 3 times weekly while grazing native range.

[3]Heifer ADG over the entire 30 d post-breeding supplementation period.

The inherently low nutritive value of dormant native forages limits voluntary intake due to gut distention, therefore supplementation is required to achieve production goals (Del Curto et al., 2000). However, animal response to supplementation can vary from predicted performance. Supplementation of animals consuming native forages has been reported to both increase and decrease forage intake. Moore et al. (1999) attributed this to the ratio of TDN to CP in the forage. Supplementation increased forage intake when the ratio of TDN:CP was > 7 and decreased forage intake when the ratio of TDN:CP was < 7. In the current study clipped forage samples had a TDN:CP ratio > 7 over the entire study, indicating that supplementation would potentially increase forage intake (data not shown). However, the TDN:CP ratio of masticate samples was < 7 for all samples except March 1, which had a TDN:CP ratio of 7.28. These data suggest supplementation decreased forage intake, altering predicted performance. Therefore, heifers potentially experienced a substitution effect, replacing forage with supplement at a greater rate than anticipated.

Influence of pre-breeding heifer development system on reproductive parameters is reported in Table 2. There were no differences in AFC, RTS or proportion of heifers attaining puberty prior to the breeding season ($P \geq 0.53$). Clanton et al. (1983) reported heifers

developed utilizing a late gain, even gain, and early gain development strategy had a similar age at first estrus between treatments. Additionally, estrus response did not differ ($P = 0.67$) between CG and LoHi heifers.

Post-breeding heifer performance

Heifer BW, ADG, and reproductive performance for the post-breeding supplementation period are reported in Table 2. Heifer post-breeding initial, mid, and final BW were similar ($P \geq 0.33$) between CON and High heifers. Similarities in performance over the post-breeding supplementation period may be attributed to animal diet selectivity. A substitution effect may have been experienced by High heifers, similar to that hypothesized during the pre-breeding period. This potential substitution effect may have decreased performance of High heifers, negating the desired effect of increasing nutritive intake as a means to increase BW gains.

Previous research has reported heifers on a decreased plane of nutrition immediately following AI had reduced pregnancy rates and embryo development compared to heifers feed at or above maintenance (Hill et al., 1970; Perry, 2012). Perry (2012) reported heifers developed in a drylot from weaning to breeding and placed on spring forage immediately following AI had reduced AI pregnancy rates compared to contemporaries placed on spring forage and supplemented 2.2 kg $\cdot$heifer$^{-1}\cdot$d^{-1} of DDGS for 42 d. In the current study, there was a trend for CON heifers to have an increased ($P = 0.10$) AI pregnancy rate compared with High supplemented contemporaries. Although post-breeding ADG between treatments was not statistically different, a numerical difference existed, with High heifers gaining 0.16 kg/d less than CON heifers. These data suggest a possible substitution effect for High heifers, replacing forage with supplement. During the 30 d period of post-breeding supplementation heifers were selecting a diet with a crude protein between 13.6% and 11.6%, which was 8.4% and 5.4% greater than crude protein in clipped forage samples. Supplementation rates were formulated to achieve desired gains based on forage values derived from clip samples using the NRC model (NRC, 2000). Selection of a higher quality diet by heifers impacted heifer BW gains, potentially altering reproductive performance.

IMPLICATION

Accurately predicting forage intake and performance of grazing animals is a major challenge for producers. Increasing the understanding of diet selectivity of cattle grazing native range could alter supplementation strategies for producers, improving their ability to meet nutrient requirements of animals, ultimately increasing performance and efficiency. Performance during the current study reflected challenges of predicting heifer performance in a grazing situation. While heifer performance did not match predicted performance, a trend for increased AI pregnancy rate was reported. Therefore, despite differences in rates of supplementation, animal diet selectivity and nutrient intake potentially impacted pregnancy success, establishing nutritional management postbreeding as a critical time point.

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Locomotor activity changes in primiparous and multiparous beef cows during the final 72 hours prepartum

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ABSTRACT: The objective of this study was to determine changes in locomotor activity of primiparous and multiparous beef females during the final 72 h prepartum. IceQube activity monitors (iceRobotics, Edinburgh, UK) were placed above the left hind fetlock of 81 spring-calving beef cows (269 ± 0.2 d of gestation) housed in 18 × 61 m drylots. Cows that were moved outside of their normal patterns during the 72 h prepartum or had unknown calving times were removed from the dataset, resulting in 13 primiparous and 22 multiparous (average parity = 4.7 ± 0.6) females. Each cow's motion index, standing time, lying time, step count, and number of lying bouts were summed per hour using IceManager 2012 software. Hour 0 was defined as time of parturition (± 30 min). Data were analyzed by day (d -3 to d -1 prepartum), by 6-h period during the final 24 h prepartum, and by hour during the final 6 h prepartum using a mixed model with fixed effects of time prepartum, parity, and their interaction in the model. Motion index, standing time, step count, and number of lying bouts increased ($P < 0.001$) while lying time decreased ($P \leq 0.003$) on d -1 compared with d -2 and -3 prepartum. Primiparous dams had greater ($P = 0.004$) standing time and less ($P = 0.004$) lying time than multiparous dams during the final 72 h prepartum. The interaction of day × parity affected ($P = 0.02$) lying bouts. In the 24 h prepartum, dams had greater ($P < 0.05$) motion index, standing time, step count, and number of lying bouts and less ($P = 0.02$) lying time during 6 h preceding parturition compared with the other 6-h periods before calving. Primiparous dams had a greater ($P = 0.004$) number of lying bouts than multiparous dams during the 24 h prepartum. Number of lying bouts tended to be greater ($P = 0.09$) in primiparous dams during the final 6 h prepartum. Motion index was greater ($P \leq 0.001$) during the final 3 h. Step count was greater ($P < 0.02$) in -1 and -2 h prepartum than -3, -4, and -5 h. Lying bout numbers increased ($P \leq 0.001$) from -3 to -2 h and -2 to -1 h before parturition. These data suggest that locomotive activities change over time prior to calving, with some differences occurring between primiparous and multiparous beef dams.

Key words: behavior, parity, parturition
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INTRODUCTION

In 2010, 20.9% of non-predator beef calf death loss was due to calving problems (USDA-APHIS, 2010). Individual monitoring of heifers and cows is required to identify calving difficulties as early as possible, allowing for human intervention to minimize the negative impacts of dystocia and prolonged calving on calf survival. Experienced cattlemen often use qualitative physical and behavioral changes to identify animals close to parturition. Continuous monitoring during calving is not a compatible management system for most producers due to the high economic or opportunity cost of labor. Early recognition of parturition in beef heifers and cows via remote sensing can assist in reducing calf mortality caused by dystocia, and is of increasing interest in some segments of the beef industry.

Electronic activity monitoring has been used in the dairy industry for health and estrus detection allowing for the observation of behavior on multiple cows without the need to physically observe each cow individually. Studies using free-stalled dairy cows indicate an increase in the number of standing bouts on the day of calving (Huzzey et al., 2005) and the greatest increase of lying frequency during the final 6 h prepartum (Miedema et al., 2011). These

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indications of increased activity prior to calving could be useful in detecting when heifers and cows are close to calving in beef cows. Our lab previously reported a change in locomotor activity from 4 to 6 h prior to parturition in multiparous beef cows housed in drylots (Bolen et al., 2016), indicating that electronic activity monitoring may also be relevant in beef cattle.

Recent data reported a difference in activity levels between primiparous and multiparous dairy cows (Titler et al., 2015). This suggests that behavioral differences associated with parity may impact use of electronic activity monitoring, but to our knowledge, the effects of parity on locomotor activity near calving have not been studied in beef cattle. We hypothesized that both multiparous and primiparous dams would have increased activity close to calving, with primiparous dams being more active near parturition. Our specific objective was to determine differences between activity levels of primiparous and multiparous dams in the final 3 d prior to parturition, final 24 h prior to parturition, and final 6 h prior to parturition.

MATERIALS AND METHODS

All procedures were approved by the University of Missouri Animal Care and Use Committee. Spring-calving crossbred beef females (n = 22 primiparous; n = 59 multiparous) were moved to six 18 × 61 m drylots for observation during calving. Dams were penned in groups of 12 to 15 animals by prior management group (parity 1 and 2 vs. parity ≥ 3). Animals were allowed ad libitum access to endophyte-infected tall fescue hay (6.7% CP and 63.9% NDF, DM basis) in round bale feeders placed near the center of drylots and fed 1.0 kg DM/d of dried distillers grains with solubles at approximately 1700 h daily. Automatic waterers were located at the front of pens near feed bunks. Pens were well-lit, and lights were kept on during the night to allow for observation of calving. Dams were walked through by personnel to monitor for physical signs of labor at least once per hour from 0600 to 2400 h, with additional monitoring between 0000 and 0600 h during heavy calving. Cows were monitored continuously from the time of visible evidence of stage 2 parturition, and actual time of birth was recorded for each calf. Minimal interference occurred during parturition except to assist as needed if there were concerns of dystocia. No dystocia requiring assistance were included in the dataset (n = 1).

One IceQube activity monitor (iceRobotics, Edinburgh, UK) was placed above the left hind fetlock of each pregnant cow or heifer monitored for calving per manufacturer instructions on 6.7 ± 2.9 d prior to calving. This activity monitoring sensor provides reliable information about the animal's position (vertical and horizontal), the movement of the leg on which it is located, and the number of steps taken by the cow (Nielsen et al., 2010). During the observation period, 59 artificially inseminated dams calved. Data were not used for cows that calved prior to IceQube placement or with unknown calving times, resulting in 35 cows to be used for analysis (Table 1). Individual day data were removed for cows moved outside of their normal patterns during the final 72 h prepartum. Movement outside normal patterns constituted cows that left pens during the 72 h period prior to calving or cows that were moved into a covered calving pen during cold weather.

TABLE 1. Parity, body weight, body condition score, and gestation length for dams on study[1]

Variable	Primiparous	Multiparous
n	13	22
Parity	1 ± 0	4.7 ± 0.6
Prepartum BW, kg	553 ± 12.3	676 ± 14.5
BCS	4.9 ± 0.1	5.4 ± 0.1
Gestation length, d	277 ± 0.6	276 ± 0.6

[1]Least square means ± SD are presented.

IceQube activity monitors were removed > 2 d postpartum. IceManager 2012 software (iceRobotics, Edinburgh, United Kingdom) was used to obtain and sum each cow and heifer's motion index, standing time, lying time, step count, and number of lying bouts per hour, then exported to an Excel spreadsheet (Microsoft Corp., Redmond, WA). Motion index was calculated by the IceManager software using a proprietary algorithm. Hour 0 was defined as the hour in which parturition occurred (± 30 min).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) in 3 separate analyses: by day during the final 72-h prepartum (d -3, -2, and -1), by 6-h period during the final 24 h prepartum (h -23 to -18, h -17 to -12, h -11 – 6, and h -5 to 0), and by hour during the final 6 h prepartum (h -5, -4, -3, -2, -1, and 0). For each analysis, the fixed effects of time prepartum, parity, and their interaction were included in the model. Animal was the experimental unit. Least squares means were separated using least significant difference and considered significant when $P \leq 0.05$. Tendencies were considered to be when $0.05 < P \leq 0.10$. In the absence of interactions, main effects of time and parity are reported.

RESULTS

Final 72 h Prepartum

The interaction of day × parity did not affect ($P > 0.81$) motion index, standing time, lying time, or

TABLE 2. Locomotor activity during the 72 h prior to calving in primiparous and multiparous beef cows

	Day				Parity			*P*-value		
Item	-3 d	-2 d	-1 d	SEM	1[1]	≥2[2]	SEM	Day	Parity	Day by Parity
Motion Index	5,747[b]	5,701[b]	8,344[a]	575	6,944	6250	500	<0.001	0.28	0.83
Standing Time, min	780.7[b]	779.2[b]	912.0[a]	18.2	853.7	794.2	15.9	<0.001	0.004	0.84
Lying Time, min	659.3[a]	660.8[a]	528.0[b]	18.2	586.3	645.8	15.9	<0.001	0.004	0.84
Step Count	1,425[b]	1,430[b]	2,093[a]	134	1,716	1,593	116	0.003	0.38	0.81
Lying Bouts	-	-	-	-	-	-	-	<0.001	0.002	0.02
Primiparous	11.00[z]	9.38[z]	25.15[x]	1.77	-	-	-	-	-	-
Multiparous	8.61[z]	9.20[z]	19.10[y]	0.93	-	-	-	-	-	-

[a,b]Within an item, main effect means differ ($P \leq 0.05$).

[x,y,z]Within an item, interactive means differ ($P \leq 0.05$).

[1]Primiparous n = 13.

[2]Multiparous n = 22.

TABLE 3. Locomotor activity during the 24 h prior to calving in primiparous and multiparous beef cows

	Time Period					Parity			*P*-value		
Item	-23 to -18 h	-17 to -12 h	-11 to -6 h	-5 to 0 h	SEM	1[1]	≥2[2]	SEM	Hour	Parity	Hour by Parity
Motion Index	1,684[b]	1490[b]	1,383[b]	3,783[a]	217	2,187	1,983	171	<0.001	0.35	0.52
Standing Time, min	236.6[ab]	212.8[b]	206.7[b]	253.3[a]	11.8	236.1	218.6	9.3	0.02	0.14	0.12
Lying Time, min	123.4[ab]	147.2[a]	153.3[a]	106.7[b]	11.8	124.0	141.4	9.3	0.02	0.14	0.12
Step Count	428.4[b]	375.1[b]	358.1[b]	930.4[a]	59.7	540.4	505.5	42.5	<0.001	0.51	0.43
Lying Bouts	2.131[b]	2.657[b]	3.467[b]	13.897[a]	0.585	6.289	4.788	0.402	<0.001	0.004	0.12

[a,b]Within an item, main effect means differ ($P \leq 0.05$).

[1]Primiparous n = 13.

[2]Multiparous n = 22.

step count when analyzed by day during the final 72 h (Table 2). Parity did not affect ($P > 0.28$) motion index or step count. There was an effect of day ($P < 0.001$) for motion index, standing time, lying time, and step count. Motion index was greater ($P < 0.001$) on d -1 than d -2 and -3. Dams spent a greater ($P \leq 0.001$) time standing on d -1 than d -2 or -3. There was also an effect parity ($P = 0.004$) on standing time, Where primiparous dams spent greater ($P = 0.004$) time standing than multiparous dams during this period. Because standing and lying are inversely related, dams spent less ($P \leq 0.001$) time lying on d -1 than d -2 or -3, and primiparous dams spent less ($P = 0.004$) time lying than multiparous dams. Dams had a greater ($P < 0.001$) step count on d -1 than d -2 or -3. The interaction of day × parity affected ($P = 0.02$) lying bouts. Females had a greater ($P \leq 0.001$) number of lying bouts on d -1 compared with d -2 and -3. Within d -1, primiparous dams had a greater ($P = 0.002$) number of lying bouts than multiparous dams.

Final 24 h Prepartum

The interaction of hour × parity did not affect ($P > 0.12$) motion index, standing time, lying time, step count, or lying bouts when analyzed by 6-h periods (h -23 to -18, h -17 to -12, h -11 to -6, and h -5 to 0) during the final 24 h (Table 3). Parity did not affect ($P > 0.14$) motion index, standing time, lying time or number of steps. There was an effect of hour ($P < 0.02$) on motion index, standing time, lying time, step count, and number of lying bouts. Motion index was greater ($P \leq 0.001$) during the final 6 h prepartum than the previous 6-h periods. Time spent standing was then greater ($P = 0.002$) from h -5 to 0 than h -11 to -6 and h -17 to -12. Time spent lying worked inversely of time spent standing and was less ($P = 0.002$) from h -5 to 0 than h -11 to -6 and h -17 to 12. Number of steps taken were greater ($P \leq 0.001$) in the 6 h prior to parturition than all other periods. Number of lying bouts was greater ($P \leq 0.001$) during the final 6 h than the h -11 to -6, h -17 to -12, and h -23 to -18 periods. There was also an effect of parity ($P = 0.004$) on lying bouts, where primiparous dams had a greater number ($P = 0.004$) of lying bouts than multiparous cows during the final 24 h prepartum.

Final 6 h Prepartum

The interaction of day × parity did not affect ($P > 0.63$) motion index, standing time, lying times, or step count during the final 6 h of parturition (Table 4). There

TABLE 4. Locomotor activity during the 6 h prior to calving in primiparous and multiparous beef cows

	Hour							Parity			*P*-value		
Item	-5 h	-4 h	- 3 h	-2 h	-1 h	-0 h	SEM	1[1]	≥2[2]	SEM	Hour	Parity	Hour by Parity
Motion Index	410.0[b]	398.3[b]	526.9[b]	817.0[a]	877.0[a]	788.4[a]	86.3	623.8	648.8	55.4	<0.001	0.72	0.97
Standing Time, min	43.92	44.80	42.35	45.96	39.39	37.41	2.72	40.91	43.70	1.75	0.20	0.21	0.63
Lying Time, min	16.08	15.20	17.65	14.04	20.61	22.58	2.72	19.09	16.30	1.75	0.20	0.21	0.63
Step Count	108.9[c]	103.3[c]	136.8[bc]	208.7[a]	208.2[a]	170.8[ab]	21.1	150.3	161.9	13.5	<0.001	0.50	0.96
Lying Bouts	0.519[c]	0.726[c]	1.199[c]	2.203[b]	4.813[a]	4.528[a]	0.354	2.577	2.086	0.227	<0.001	0.09	0.81

[a,b,c]Within an item, main effect means differ ($P \leq 0.05$).

[1]Primiparous n = 13.

[2]Multiparous n = 22.

was no effect ($P > 0.21$) of parity for motion index, standing time, lying time or step count. There was an effect of hour ($P < 0.001$) on motion index, step count and lying bouts. Motion index was greater ($P \leq 0.001$) during 0, -1, and -2 h prepartum compared with -3 to -5 h. There was no effect of parity ($P = 0.72$) on motion index. Steps were greater ($P < 0.02$) for h -1 and -2 than h -3, -4 and -5. Lying bouts increased ($P \leq 0.001$) from h -3 to -2 and h -2 to -1. Primiparous dams tended to have a greater ($P = 0.09$) incidence of lying bouts than multiparous dams during the final 6 h prior to parturition.

DISCUSSION

To date, limited research involving the locomotor activity prior to calving has been conducted in beef cattle. An understanding of how maternal activity changes near parturition may enable use of quantitative behavioral signs to predict time of calving. Previous research from our laboratory suggest that electronic activity monitors could be used to recognize the earliest signs of parturition in beef cattle because of changes in activity 4 to 6 h prior to parturition (Bolen et al., 2016).

Primiparous cows spent more time standing and less time lying than multiparous cows on the day prior to parturition in the current study. This is contradictory to previous data in dairy cows, where primiparous cows spent less time standing (Titler et al., 2015). Additionally, primiparous dairy cows spent more of the final 2 h prepartum lying down compared with multiparous cows in another study (Miedema et al., 2011). This was attributed to primiparous dams spending more time in labor and having more contractions during parturition (Miedema et al., 2011). This is contrary to our data where there was no difference between parity at any hour during the 6-h period immediately prior to parturition. Our data demonstrated a decrease in lying time during the final 6-h period when compared to -24 to -19 h, -18 to -13 h, and -12 to -7 h periods which further contradicted dairy cow data (Miedema et al., 2011). These differences between studies could be attributed to different activities of beef cattle, or because of variation in locomotor data collection. Standing time could still be helpful in determining when cows are near calving and be beneficial to producers that may like to move cows to a more confined area to observe calving.

Previous research with dairy cattle has shown that electronic data loggers detected an increase in number of steps within the 24 h immediately prior to parturition (Titler et al., 2015). Our data support the increase in number of steps during the final day when compared with 3 and 2 d prior to parturition. Our current study narrowed the time frame to an increase in step number specifically occurring between h -3 and -2 and then again from h -2 to -1 prepartum. Previous research in our laboratory supports this (Bolen et al., 2016). This increase in steps could be due to females walking while searching for a safe place to calve or pacing due to discomfort.

An increase in number of lying bouts within the final 24 h prior to parturition has also been previously shown in dairy cattle using electronic data loggers (Titler et al., 2015). Our data support the increase when compared with 3 and 2 d prior to parturition. This is supported by previous research in beef cows (Bolen et al., 2016). During the final 24 h, primiparous dams had an increased number of lying bouts when compared with multiparous dams. Number of lying bouts increased from h -3 to -2 and again from h -2 to -1. The mean number of lying bouts more than doubled from h -2 to -1. This fact, combined with the decreased amount of time spend lying during the 24 h immediately prepartum, suggests that primiparous dams are more restless and change their activity from lying to standing more frequently resulting in shorter, more frequent lying bouts.

The motion index algorithm was developed to indicate the total amount of activity, considering the previously discussed factors, where a higher motion index indicates that cattle are more active. In the current study,

motion index increased during the day prior to parturition. Further analysis of our current data demonstrates that the general activity of females increased 3 h prior to calving. This increase was likely attributed to the activity of changing standing and lying status frequently as seen with the increase in number of lying bouts. There was no difference between multiparous and primiparous dams, suggesting that the increase in activity occurs independent of parity. Motion index could be useful in more closely identifying an increased level of activity in order to predict parturition within a narrower window of time.

In conclusion, the current study demonstrates that locomotor activity is elevated during the 24 h prior to calving in beef cattle. This increased activity was most notable during the final 3 h. More research in this area may allow for development of methods for detecting calving via activity monitoring technology.

IMPLICATIONS

Beef cows and heifers approaching parturition display increased movement during the final 24 h prior to calving that can be detected with electronic activity monitors such as IceQubes. Locomotor activity monitoring may be an opportunity to optimize precision calving management, helping to decrease calf mortality or neonatal morbidity due to dystocia.

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Estimation of genetic parameters and effects of birth type, sex, and age of dam on lamb mortality[1,2]

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ABSTRACT: Lamb mortality has a significant impact on economic loss in the sheep industry. In this study, lamb mortality was evaluated two ways: 1) all mortality prior to weaning (i.e., preweaning mortality) and 2) mortality at birth (or when found). Mortality was evaluated assuming a binomial distribution and logit link function using generalized linear mixed models. Bonferroni correction for multiple tests was applied for significance at $\alpha = 0.05$ ($\alpha/n_{tests} = 4.39 \times 10^{-4}$). Males had greater ($P < 0.001$) preweaning mortality, but there was no sex difference detected for mortality at birth ($P = 0.043$). Preweaning lamb mortality was greater for very young ewes (1 year old), but mortality at birth was greater for both very young (1.5 years or less) and very old ewes (8 and 9 years old). The estimate of heritability for preweaning mortality was 0.123 ± 0.016, which was lower than mortality at birth: 0.241 ± 0.027. As a proportion of phenotypic variance, maternal additive genetic effects were 0.105 ± 0.020 and 0.082 ± 0.039 for preweaning mortality and mortality at birth, respectively. Furthermore, maternal permanent environmental effects were 0.025 ± 0.016 and 0.286 ± 0.032, and estimates of correlation of additive genetic-maternal additive genetic effects were -0.026 ± 0.140 and -0.344 ± 0.178. Preweaning mortality results were consistent with other similar work in sheep; however, mortality at birth results were quite different and merit additional exploration. Heritability estimate of additive genetic effects for mortality at birth were almost double the estimate for preweaning mortality. Genetic evaluation could be accelerated and selection implemented with more impact using breeding values for mortality at birth.

Key words: crossbreeding, genetic parameters, lamb mortality
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INTRODUCTION

Lamb mortality has a significant impact on economic loss in the sheep industry. According to the National Agricultural Statistics Service, it was estimated that 63% (or 400,000 head) of the total loss of sheep in 2009 were lambs and these deaths accounted for approximately a $25 million loss (NASS, 2010). Cause of death is generally not considered when estimating genetic parameters for lamb mortality (e.g., Southey et al., 2004). However, genetic variation may differ for distinct causes of death. This has been observed by Gama et al. (1991) for different causes of preweaning death between perinatal, postnatal, respiratory, digestive, injury, and starvation. Estimates of heritability for lamb mortality for different stages from birth to one year of age have consistently reported to be very low, from 0.0 to 0.1 (Southey et al., 2001). The objective of this study was to estimate genetic parameters associated with lamb survival for preweaning mortality and mortality at birth.

MATERIALS AND METHODS

Animals

All sheep records used in this study were from the USDA, ARS, Range Sheep Production Efficiency Research Unit, U.S. Sheep Experiment Station located near Dubois, ID. Records used (n =

[1] Authors wish to thank Natalie Pierce for providing the initial data queries.
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62,113; 2000 to 2015) for this study were a subset of much larger dataset (n = 270,400) from 1950 to 2015 as a preliminary investigation of genetic parameter estimates. In this subset, records included a large number of purebred and crossbred animals, where breeds represented included Columbia, Hampshire, Merino, Polypay, Rambouillet, Suffolk, Targhee, Friesian, Dorper, Texel, Merino, and a USDA MARC composite (M3; 0.5 Columbia 0.25 Hampshire 0.25 Suffolk).

Statistical Analyses

Lamb death was evaluated assuming a binomial distribution and a logit link function using generalized linear mixed models with ASREML (Gilmour et al., 2015) using all records. Fixed effects investigated included lamb breed, dam breed, the type of birth (i.e., single, twin, triplet, quadruplet/quintuplet), sex of the lamb (male, female, unknown), the age of the dam (1 to 7 in 0.5 year categories as well as ages 8 and 9), and the year of record. Random effects considered included additive genetic, maternal additive genetic, and the covariance of those, as well as maternal permanent environmental effects. Bonferroni correction for multiple tests were used for $\alpha = 0.05$ ($\alpha/n_{tests} = 4.39 \times 10^{-4}$; Bland and Altman, 1995). Pedigree information was used to confirm breed designations for each individual animal. Many lambs that died at birth did not have sex recorded for a variety of reasons, which created the unknown category for sex effect. Wethers and rams were combined into a single sex category. In the first analysis (preweaning mortality), lambs that were alive at weaning were given a value of 0; lambs that died or were removed for other reasons were given values of 1 (n = 17,722). In the analysis of birth mortality, any lamb dead at birth was given a value of 1 (n = 5,173), and those alive were given values of 0.

RESULTS AND DISCUSSION

Overall lamb mortality prior to weaning in this study was 28.5%. Of those deaths the most frequent cause for removal from the flock prior to weaning was lambs that were born dead. Lambs born dead had a mortality rate of 8.3% which made up 29.2% of all deaths prior to weaning.

Many animals used were crossbred of varying breeds and fractions within those breeds. This created a very large set of lamb and dam breed categories. Additionally, the large correspondence between levels of these two effects (i.e., lamb breed vs. dam breed) prevented them from being included in models simultaneously. Ultimately, they were both removed in order to achieve appropriate convergence and parameter estimation in the equations. An important next consideration for work with these data is to more concisely describe breed types from a modeling perspective.

TABLE 1. Means for all levels of fixed effects for preweaning mortality and mortality at birth

	Trait	
Fixed Effect	Preweaning Mortality	Birth Mortality
Type of birth		
Single	$0.615^{a} \pm 0.038$	$0.182^{a} \pm 0.018$
Twin	$0.781^{b} \pm 0.028$	$0.228^{b} \pm 0.020$
Triplet	$0.920^{c} \pm 0.013$	$0.420^{c} \pm 0.029$
Quadruplet[1]	$0.953^{d} \pm 0.008$	$0.543^{d} \pm 0.040$
Sex		
Ram	$0.355^{a} \pm 0.011$	$0.046^{a} \pm 0.005$
Ewe	$0.332^{b} \pm 0.011$	$0.050^{a} \pm 0.005$
Unknown	$0.999^{c} \pm 0.001$	$0.979^{b} \pm 0.004$
Age of dam		
1	$0.945^{a} \pm 0.009$	$0.605^{a} \pm 0.021$
1.5	$0.884^{b} \pm 0.029$	$0.282^{a} \pm 0.095$
2	$0.872^{b} \pm 0.018$	$0.383^{b} \pm 0.019$
2.5	$0.821^{b} \pm 0.037$	$0.273^{b} \pm 0.070$
3	$0.816^{b} \pm 0.024$	$0.300^{b} \pm 0.017$
3.5	$0.803^{b} \pm 0.039$	$0.281^{b} \pm 0.072$
4	$0.807^{b} \pm 0.025$	$0.277^{b} \pm 0.016$
4.5	$0.837^{b} \pm 0.031$	$0.247^{b} \pm 0.060$
5	$0.810^{b} \pm 0.025$	$0.255^{b} \pm 0.017$
5.5	$0.802^{b} \pm 0.040$	$0.287^{b} \pm 0.070$
6	$0.828^{b} \pm 0.023$	$0.314^{b} \pm 0.020$
6.5	$0.852^{b} \pm 0.034$	$0.393^{b} \pm 0.075$
7	$0.850^{b} \pm 0.021$	$0.310^{b} \pm 0.023$
8	$0.913^{b} \pm 0.015$	$0.512^{a} \pm 0.044$
9	$0.916^{b} \pm 0.037$	$0.270^{a} \pm 0.160$

[a-d]Means in column for each fixed effect that have the same superscript do not differ ($P < 4.39 \times 10^{-4}$) after correction for multiple tests.

[1]Quadruplets grouped together with quintuplets, records did not allow for distinction between them.

Fixed Effect Means

Means were calculated for all levels of the fixed effects (Table 1). For both traits, there were significant differences between the types of birth (i.e., single, twin, triplet, quadruplet/quintuplet) related to lamb mortality. It has been reported that larger birth types have a significantly greater chance of mortality (Southey et al., 2001, 2004) and the chances of survival to weaning are greater in single lambs (Álvarez et al., 2010). Differences in sex were seen in relation to any deaths prior to weaning with males greater than females ($P = 0.001$), however there was no difference detected between ram and ewe offspring for lambs born dead ($P = 0.043$) after Bonferroni correction. Both did differ from the unknown sex category and is most likely caused by sex not being recorded on indi-

viduals that were born dead. A large portion (41.3%) of the animals that were born dead did not receive sex classifications and 96.1% of the unknown-sex lambs were dead at birth or born premature. There were differences seen in age of the dam as well. Dams at 1 year of age experienced greater preweaning mortality compared to all other age groups ($P < 0.001$). No differences were seen between 1, 1.5, 8, and 9 years of age for lambs dead at birth. There were other small differences observed for both traits on age of dam, but most likely due to standard errors.

Estimation of Genetic Parameters

The random structure most favored by log-likelihood values was the full model which included the additive genetic, maternal additive genetic, the covariance between them, and the maternal permanent environmental effects. Genetic parameters and variances are shown in Tables 2 and 3. Large changes between traits were seen in the additive variance, 0.163 to 0.550, the maternal permanent environmental variance, 0.033 to 0.650, and the covariance between these two, -0.004 to -0.110. These changes are likely due to the distribution of the traits. Lambs that were born dead only accounted for 29.2% of the total deaths. Heritability for lamb survival has been previously estimated from 0.00 to approximately 0.10 (Southey et al., 2001). The heritability estimates for preweaning mortality were similar to previous studies. Also the correlation between the additive and maternal effects was negative but still close to zero. For mortality at birth, the heritability estimates were greater than expected. Previous heritability estimates for early lamb survival have been estimated to be 0.011 for additive and 0.006 for maternal with inclusion of the permanent environmental effect of the dam (Everett-Hincks et al., 2014). In this study along with the variance component of permanent environmental effect of the dam, the additive and maternal covariance was accounted for as well. The correlation coefficient for the additive and maternal effects were moderately negative, -0.344 ± 0.178.

TABLE 2. Estimates of variance for preweaning and birth mortality

	Trait	
Parameter	Preweaning Mortality	Birth Mortality
Additive variance	0.163 ± 0.023	0.550 ± 0.069
Maternal variance	0.139 ± 0.027	0.187 ± 0.088
Covariance between additive and maternal	-0.004 ± 0.021	-0.110 ± 0.073
Maternal permanent environmental variance	0.033 ± 0.022	0.650 ± 0.791

TABLE 3. Estimates of genetic parameters for preweaning and birth mortality

	Trait	
Parameter	Preweaning Mortality	Birth Mortality
Additive heritability	0.123 ± 0.016	0.241 ± 0.027
Maternal heritability	0.105 ± 0.020	0.082 ± 0.039
Maternal permanent environmental heritability	0.025 ± 0.016	0.286 ± 0.032
Correlation between additive and maternal	-0.026 ± 0.140	-0.344 ± 0.178

IMPLICATIONS

Estimates of additive heritability were almost twice as high for mortality at birth compared to preweaning mortality and there was an even larger change in the correlations for the two traits. Genetic evaluation could be accelerated and selection implemented with more impact using breeding values for mortality at birth. These results will be used as a starting point and the results will be taken into consideration for further research with the full dataset. Parameterization of breed type merits additional consideration as well as levels of age, as there were little differences between specific ages.

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Wool characteristics of Rambouillet, Polypay, and Romanov-White Dorper x Rambouillet ewes in an extensive rangeland production system

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ABSTRACT: The objectives of this study were to quantify and compare wool characteristics in Rambouillet (RA), Polypay (PP), and Romanov-White Dorper x Rambouillet (RW-RA) ewes under extensive rangeland management conditions. Side samples were collected from 678 ewes at 1 and 4 yr of age and objective fiber characteristics were measured on a total of 1,074 samples. Clean fleece weights of 1-yr-old ewes were not different ($P > 0.90$) among breed types. Four-yr-old RA ewes had heavier clean fleeces ($P < 0.01$) than 4-yr-old PP and RW-RA ewes, which were not different ($P > 0.95$). Within 1- and 4-yr-olds, RA fleeces were finest (20.1 ± 0.11 μm and 21.9 ± 0.14 μm, respectively), RW-RA fleeces were intermediate (22.8 ± 0.11 μm and 24.9 ± 0.13 μm), and PP fleeces were coarsest (24.3 ± 0.11 μm and 26.8 ± 0.14 μm), ($P < 0.01$). Standard deviation of fiber diameter was lowest in 1- and 4-yr-old RA fleeces (4.22 ± 0.09 μm and 4.14 ± 0.10 μm, respectively), intermediate in PP (5.16 ± 0.08 μm and 5.19 ± 0.10 μm), and greatest in RW-RA (7.05 ± 0.08 μm and 6.55 ± 0.09 μm), ($P < 0.01$). Breed types within age differed ($P < 0.01$) in curvature; RA wool had the largest values, PP wool was intermediate, and RW-RA wool had the smallest values. Wool from RW-RA ewes had more kemp and more medullated fibers ($P < 0.01$) than wool from RA and PP ewes, which were not different ($P > 0.95$). Results indicated superior wool production and most desirable quality characteristics for RA ewes compared to coarser, more variable wool produced by PP and RW-RA ewes. Higher proportions of kemp and medullated fibers were found in in RW-RA fleeces, but these fleeces were estimated to be within acceptable thresholds for a given micron range and, under conditions similar to this study, would be marketable.

Key words: crossbred, fiber diameter, kemp fibers, wool
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INTRODUCTION

Rangeland sheep production businesses rely on the sale of lamb and wool to increase gross receipts. From 2010 to 2015, wool represented an estimated 6 to 12% of total revenue on U.S. sheep operations (LMIC, 2016), providing timely seasonal cash flow. Strategies to optimize lamb production while maintaining adequate wool quality characteristics and a marketable clip may be an attainable production benchmark. Crossbreeding using rams from prolific breeds (e.g., Finnsheep and Romanov) and traditional Western white-faced ewes, or the use of semi-prolific composite breeds such as the Polypay, increases the number and weight of lamb weaned per ewe (Young et al., 1996). Similarly, development of hair sheep composite breeds such as the Katahdin and Dorper have increased lamb production and survival (Notter, 1999), although crossbreeding hair breeds with wool breeds resulted in increased kemp

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and medullated fiber content in F1 progeny, thereby limiting wool marketability (Balasingam, 2005). Information regarding kemp and medulated fiber content in ¼-hair sheep crossbreds is not widely reported. Specifically, fleece characteristics of Romanov-White Dorper x Rambouillet ewes are unknown and merit investigation to objectively determine marketability. Worsted and woolen systems are the 2 processes that take raw wool to yarn. The worsted system uses finer, longer grades of wool for higher quality apparel products, whereas the woolen system uses coarser grades of wool for yarn production into non-apparel products (Teasdale and Cottle, 1991). Thus, the objectives of this study were to quantify and compare wool characteristics in Rambouillet, Polypay, and Romanov-White Dorper x Rambouillet crossbred ewes under extensive rangeland management conditions at the U.S. Sheep Experiment Station (USSES).

MATERIALS AND METHODS

Animals and Management

The USSES Institutional Animal Care and Use Committee approved all husbandry practices and experimental procedures used in this study (#0905). Ewes for this study were born in 2009, 2010, and 2011. In each year, lambs were produced by mating USSES Rambouillet ewes to 1 of 7 Rambouillet rams or 1 of 7 Romanov x White Dorper rams and mating USSES Polypay ewes to 1 of 7 Polypay rams all in single-sire mating pens. Dams were 2 to 8 yr old at lambing. Resulting ewe lambs were exposed to rams for the first time at 7 to 8 mo of age and retained for up to 4 lambings. Thus, ewes evaluated for wool characteristics were Rambouillet (RA), Polypay (PP), or ¼ Romanov, ¼ White Dorper, and ½ Rambouillet (RW-RA).

Wool Analysis

A total of 1,074 side samples were collected from 678 ewes at 1 and 4 yr of age and sent to the Montana Wool Laboratory at Montana State University where objective measurements of fiber traits were conducted. An Optical Fiber Diameter Analyzer 2000 (BSC Electronics Pty. Ltd., Attadale, Western Australia) was calibrated for opacity measurement using a single standard slide of known opacity provided by the instrument manufacturer. All of the side samples were cut with a guillotine to produce snippets (short pieces of fiber ≈ 2 mm in length) and then washed. These snippets were measured for average fiber diameter (AFD), SD of fiber diameter (FD-SD), comfort factor (CF), and curvature (CURV) according to the International Wool Textile Organization (2013). A subset of side samples (n = 128), distributed by breed type and age, were sent to the Bill Sims Wool and Mohair Research Laboratory, San Angelo, Texas, where medullated, flat, and objectionable fibers were quantified using an OFDA 100 (BSC Electronics Pty. Ltd., Attadale, Western Australia) according to the International Wool Textile Organization (2000). Grease fleece weights (GFW) were recorded at shearing. Ewe BW was collected each spring ~30 d after lambing. Estimated clean wool fibers present were analyzed (ASTM, 1990a) on composited side samples within breed type to calculate clean fleece weights (CFW; Table 1).

TABLE 1. Least-squares means (± SE) for the interaction of ewe age and breed type for grease fleece weight (GFW) and clean fleece weight (CFW)

Ewe age, yr	Breed type[1]	Trait	
		GFW, kg	CFW,[2] kg
1	RA	2.44 ± 0.04[a]	1.44 ± 0.02[a]
	PP	2.22 ± 0.04[b]	1.40 ± 0.02[a]
	RW-RA	2.01 ± 0.04[c]	1.40 ± 0.02[a]
4	RA	3.91 ± 0.05[a]	2.30 ± 0.03[a]
	PP	2.91 ± 0.05[b]	1.84 ± 0.03[b]
	RW-RA	2.65 ± 0.04[c]	1.87 ± 0.03[b]

[a–c]Means within column with no superscript in common are different ($P < 0.01$).

[1]RA = Rambouillet; PP = Polypay; RW-RA = ¼ Romanov, ¼ White Dorper, and ½ Rambouillet.

[2]CFW = GFW x clean wool fibers present (CLWFP), CLWFP: RA=58.9%, PP=63.1%, RW-RA=70.2%.

Statistical Analysis

Grease fleece weight and CFW were analyzed using the MIXED procedure of SAS (v9.4; SAS Inst. Inc., Cary, NC) and included fixed effects of breed type, birth year, and age along with the random effect of ewe nested within breed type. Additionally, spring BW was fit as a linear covariate in the analysis of GFW and CFW. Fiber metrology traits (AFD, FD-SD, CF, and CURV) were analyzed using the MIXED procedure of SAS and included fixed effects of breed type, birth year, and age along with the random effect of ewe nested within breed type. Because of subsampling, individual ewes usually did not have multiple percentage kemp and percentage total medullated fiber (kemp + hair) records, and these traits were analyzed using the GLM procedure and the fixed effects of breed type, birth year, and age. All possible 2-way interactions in the aforementioned models were tested and subsequently removed from the final models if they were not deemed significant ($P > 0.05$).

TABLE 2. Least-squares means (± SE) for the interaction of ewe age and breed type for average fiber diameter (AFD), SD of fiber diameter (FD-SD), comfort factor (CF), and curvature (CURV)

		Trait			
Ewe age, yr	Breed type[1]	AFD, µm	FD-SD, µm	CF, %	CURV , ° mm^{-1}
1	RA	20.1 ± 0.12^{c}	4.22 ± 0.09^{c}	98.6 ± 0.51^{a}	104.2 ± 1.00^{a}
	PP	24.3 ± 0.11^{a}	5.16 ± 0.08^{b}	88.7 ± 0.48^{b}	96.3 ± 0.94^{b}
	RW-RA	22.8 ± 0.11^{b}	7.05 ± 0.08^{a}	89.8 ± 0.48^{b}	87.7 ± 0.95^{c}
4	RA	21.9 ± 0.14^{c}	4.14 ± 0.10^{c}	96.0 ± 0.62^{a}	108.9 ± 1.16^{a}
	PP	26.8 ± 0.14^{a}	5.19 ± 0.10^{b}	76.1 ± 0.61^{c}	92.2 ± 1.13^{b}
	RW-RA	24.9 ± 0.13^{b}	6.55 ± 0.09^{a}	83.5 ± 0.55^{b}	87.1 ± 1.04^{c}

$^{a–c}$Means within column and ewe age with no superscript in common are different ($P < 0.01$).

[1]RA = Rambouillet; PP = Polypay; RW-RA = ¼ Romanov, ¼ White Dorper, and ½ Rambouillet.

RESULTS AND DISCUSSION

Grease fleece weight and CFW results are presented in Table 1. The breed type x age and birth year x age interactions were significant for both traits ($P < 0.01$). The least squares means for GFW within age differed ($P < 0.01$) among breed types; 1- and 4-yr-old RA raw fleeces were the heaviest (2.44 ± 0.04 kg and 3.91 ± 0.05 kg, respectively), PP fleeces were intermediate (2.22 ± 0.04 kg and 2.91 ± 0.05 kg), and RW-RA fleeces were the lightest (2.01 ± 0.04 kg and 2.65 ± 0.04 kg). Clean fleece weights of 1-yr-old ewes were not different ($P > 0.90$) among breed types. Within 4-yr-old ewes, RA had the heaviest CFW (2.30 ± 0.03 kg; $P < 0.01$) and the CFW of PP and RW-RA ewes were not different (1.84 ± 0.03 kg and 1.87 ± 0.03 kg, respectively; $P > 0.95$). Clean fleece weight is a major quality determinant for both worsted and woolen systems, especially because the majority of wool is traded on a clean basis (Teasdale and Cottle, 1991). Clean fleece weight values for RW-RA in the current study are similar to values for Romanov x Targhee fleeces reported by Berger and Lupton (1994).

Results for AFD and FD-SD are reported in Table 2. The breed type x age and birth year x age interactions were significant for AFD ($P < 0.01$). The least squares means for AFD within ewe age differed among breed types ($P < 0.01$); 1- and 4-yr-old RA fleeces were finest (20.1 ± 0.11 µm and 21.9 ± 0.14 µm, respectively), RW-RA fleeces were intermediate (22.8 ± 0.11 µm and 24.9 ± 0.13 µm), and PP fleeces were coarsest (24.3 ± 0.11 µm and 26.8 ± 0.14 µm). The breed type x birth year, breed type x age, and birth year x age interactions were significant for FD-SD ($P < 0.01$). Within 1- and 4-yr-old ewes, FD-SD was lowest in RA fleeces (4.22 ± 0.09 µm and 4.14 ± 0.10 µm, respectively), intermediate in PP (5.16 ± 0.08 µm and 5.19 ± 0.10 µm), and greatest in RW-RA (7.05 ± 0.08 µm and 6.55 ± 0.09 µm), ($P < 0.01$).

In addition to clean yield, AFD is a major price determinant for the worsted and woolen systems; however, fiber diameter variability (i.e., FD-SD) is of lesser importance for apparel end use in the woolen system vs. worsted system (Teasdale and Cottle, 1991). The spin count (U.S. Grade) system specifies a micron range and a corresponding maximum permissible FD-SD. Although RW-RA fleeces had finer AFD than PP fleeces, the increased FD-SD in RW-RA vs. PP fleeces would result in a greater proportion grading lower than their AFD class (i.e., 58's vs. 60's). On an individual-fleece basis, 234 out of 389 (60.2%) RW-RA fleeces, 12 out of 354 (3.4%) PP fleeces, and 57 out of 331 (17%) RA fleeces would be downgraded because of their large FD-SD. It is important to note that PP fleeces generally had greater AFD (lower spin count) and thus the maximum FD-SD for their 58's spin count was less stringent, on average, than that of a finer fleeced RA. The FD-SD characteristics in RW-RA wools were similar to values reported for Romanov x Targhee and Romanov x Northwestern Whiteface wools (Berger and Lupton, 1994; Lupton et al., 2004).

Results for CF and CURV are reported in Table 2. The breed type x age and birth year x age interactions were significant in the analysis of CF ($P < 0.01$). Within 1-yr-old ewes, RA fleeces had the greatest comfort factor (98.6 ± 0.51 %; $P < 0.01$) compared to PP (88.7 ± 0.48 %) and RW-RA (89.8 ± 0.48 %), which were not different ($P > 0.55$). The CF of all breed types differed ($P < 0.01$) in 4-yr-old ewes; RA fleeces were the highest (96.0 ± 0.62 %), RW-RA fleeces were intermediate (83.5 ± 0.55 %), and PP fleeces were the lowest (76.1 ± 0.61 %). Comfort factor is the percentage of fibers less than 30 µm. Research suggests fibers greater than 30 µm do not bend when they come in contact with the skin. A CF greater than 95% generally indicates that the wool is suitable for close-to-skin garment applications (Naylor et al., 1995; Naylor, 2009). Similar to AFD characteristics in the current study, measurements of CF indicate that RA wool is well-suited to worsted applications, whereas PP and RW-RA would be more appropriate for woolen applications (Stobart et al., 1986).

TABLE 3. Least-squares means (± SE) for the main effect of breed type for percentage kemp (KEMP) and total medullated fiber (MED)

	Trait	
Breed type[1]	KEMP, %	MED, %
RA	0.01 ± 0.05[b]	0.21 ± 0.19[b]
PP	0.01 ± 0.05[b]	0.20 ± 0.19[b]
RW-RA	0.31 ± 0.05[a]	1.44 ± 0.20[a]

[a,b]Means within column with no superscript in common are different ($P < 0.01$).

[1]RA = Rambouillet; PP = Polypay; RW-RA = ¼ Romanov, ¼ White Dorper, and ½ Rambouillet.

TABLE 4. Estimated spin count, wool value ($ kg^{-1}; clean basis), and gross revenue per fleece ($ hd^{-1}) from Rambouillet, Polypay, and Romanov-White Dorper x Rambouillet (RW-RA) wool

	Economic Measure[2]		
Breed Type[1]	Spin Count	$ kg^{-1}	$ hd^{-1}
RA	64's	8.88	21.85
PP	58's	6.43	13.00
RW-RA	58's	6.43	13.00

[1]RA = Rambouillet; PP = Polypay; RW-RA = ¼ Romanov, ¼ White Dorper, and ½ Rambouillet.

[2]Spin count= Corresponding micron range and SD from Table 2. $ kg^{-1} clean= USDA-AMS clean wool prices 3/2017, (75% Australia price). $ hd^{-1} = USDA AMS clean wool price x LSM clean fleece weight (Table 1).

The breed types x age and birth year x age interactions were significant for CURV ($P < 0.01$). Within 1- and 4-yr-old ewes, all breed types differed ($P \leq 0.01$) in CURV; RA fleeces had the greatest (104.2 ± 1.00 and 108.9 ± 1.16° mm^{-1}, respectively), PP fleeces were intermediate (96.3 ± 0.94 and 92.2 ± 1.13° mm^{-1}), and RW-RA fleeces had the least (87.7 ± 0.95 and 87.1 ± 1.04° mm^{-1}). Curvature is an indicator of the crimp pattern and has been reported to impact the efficiency of top making and spinning, and on tactile and structural properties of fabrics (Sommerville, 2002). Wools with high CURV (greater than 100° mm^{-1}) generally possess greater crimp per centimeter than medium- (60 to 90° mm^{-1}) and low-curvature (less than 50° mm^{-1}) wools. Curvature is not a pricing determinant for wool marketed in the U.S., however, buyers wishing to visually identify wool from ¼ hair-sheep such as the RW-RA might be able to do so based on its lower curvature.

Results from the analysis of percentages of kemp and medullated fiber are presented in Table 3. Wool from RW-RA had greater ($P < 0.01$) proportions of kemp fibers than wool from RA and PP, which were not different ($P > 0.95$). Similarly, proportions of medullated fibers were greatest in RW-RA fleeces ($P < 0.01$) compared to RA and PP, which were not different ($P > 0.95$). Medullated fibers are characterized by having a central canal (medulla) containing cell residues and air pockets that are both continuous or fragmented along their length. Kemp, hair, and heterotypes are all considered medullated fibers. Kemp fibers are short in length and chalky white in appearance, flat, very coarse (80 - 100 µm), and their medulla is 60% or more of their fiber diameter. Medullated fibers (hair and heterotypes) are long to intermediate in length and opaque in appearance, circular, fairly coarse (48- 60 µm), and their medulla is less than 60% of their fiber diameter (ASTM, 1990b; IWTO, 2000; Balasingam, 2005). Kemp and medullated fibers present challenges for manufacturers as they lack tensile strength, can easily break during first-stage processing, and resist dye, which creates color inconsistency in resulting fabrics (Morgan, 2003). Considerable fiber breakage of the coarsest medullated and kemp fibers can result in their removal during the carding and combing processes of the worsted system (Turpie, 1971).

Stobart et al. (1986) reported that medullated fibers were relatively common in medium-wool breeds such as the Polypay with a reported range of 0.2% to 1.6% in wool from 1- and 4-yr-old ewes, respectively. Progeny of St. Croix and Barbados rams bred to Targhee ewes had 0.88% and 1.16% kemp fibers, and 5.97% and 5.36% medullated fibers, respectively (Bunge et al., 1996). Romanov x Targhee ewes produced fleeces with 0.48% kemp fibers and 0.74% medullated fibers. In that study, 88% of the Romanov x Targhee fleeces contained kemp fibers with 58% being below the acceptable 0.5% threshold for the 25.3 to 27.0 µm range (Berger and Lupton, 1994).

In the current study, 100% of the RA and PP fleeces contained less than 0.5% kemp fibers compared with 82.5% of the RW-RA fleeces (Table 4). Assuming the 0.5% threshold cited by Berger and Lupton (1994), it's unlikely that the kemp values for RW-RA sheep in the current study represent a significant contamination risk if wool lots are properly labeled. From an Australian wool marketing perspective, Morgan (2003) mentioned that buyers acknowledge that non-Merino wools inherently pose medullated fiber risk and that a significant portion of medullated fibers will be lost due to breaking and removal during first-stage processing (top-making). A system for standardized appraisal and wool bale labeling system through the Australian Wool Exchange can characterize the visual degree of kemp contamination. However, the rapid, pre-sale medullated fiber test has not been widely adopted in Australia, nor are defined threshold limits established in raw wool trading. Similarly, kemp and medullated fiber testing in the U.S. is underutilized (A. McColl, Denver, CO, personal communication), forcing buyers and sellers to rely on error-prone subjective assessments.

Implications

Results indicated superior wool production and most desirable quality characteristics for RA ewes compared to coarser, more variable PP and RW-RA ewes (Table 4). Based off current (March, 2017) U.S. clean wool trade, values from the present study indicate that gross revenue per fleece are similar for PP and RW-RA. However, RW-RA should be marketed for woolen (vs. worsted) processing applications. Although RW-RA wool contained a greater proportion of kemp fibers, it's noteworthy that 82.5% of fleeces would fall below the accepted 0.5% threshold, which indicates that RW-RA wool is marketable. Although not a component of the data presented herein, historical data suggests it's unlikely that the additional revenue from RA wool would compensate for the production improvements in number and weight of lamb weaned per ewe in prolific and semi-prolific breeds and crossbreds. Thus, selection and crossbreeding decisions should continue to prioritize the improvement of lamb production and ewe longevity with a secondary emphasis on fleece traits.

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The effect of precipitation received during gestation on progeny performance in Bos indicus–influenced beef cattle

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ABSTRACT: The objective of this study was to determine the effect of precipitation level during key production time points in utero on beef progeny performance. The hypothesis was precipitation level during different periods of gestation would program calves for an environment similar to that experienced in utero resulting in altering growth and reproductive performance of calves. Data were collected on Brangus cows (n = 2,429) over a 46 yr span at the Chihuahuan Desert Rangeland Research Center (CDRRC). Recorded precipitation values were utilized to calculate average precipitation associated with total (April-April), monsoon (July-September), and late gestation (December-February). These values were used to classify treatments: low (z-value ≤ -1.00), average (z-value -0.99 - +0.99), and high (z-value ≥ +1.00) for each time period. Mixed models were used to evaluate the amount of precipitation received during different gestational time frames on subsequent calf performance. Calves experiencing high precipitation throughout gestation had heavier birth BW (P = 0.02), weaning BW (P = 0.05), and adjusted 205 d BW (P = 0.04) than low calves. Female progeny experiencing low precipitation throughout gestation were more likely to remain (P < 0.0001) in the herd and calve after the age of 8 compared with heifers experiencing high precipitation levels in utero (38% vs. 16% ± 5%, respectively). Additionally, heifers experiencing low precipitation levels during the monsoon period resulted in a greater percentage (P < 0.0001) of females calving after the age of 8 yr. Similarly, low treatment calves during those same time points also had a greater number of calves while in production (P < 0.0001) when compared to the average and high treatment groups. These results indicate that selection of heifers exposed to lower than average precipitation levels in utero may result in increased herd retention and productivity.

Key words: beef, fetal programming, longevity, precipitation
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INTRODUCTION

Cattle producers are dependent on adequate precipitation for sustaining herds. Nutrient composition of range pastures fluctuates with time of year and annual precipitation (Murphy, 1970; Marshal et al., 2005). Variability in precipitation can cause negative effects on forage growth and quality (Oelberg, 1956). Forages undergo a translocation process under normal precipitation circumstances that pulls nutrients from the root network into the stem and leaf system dictating mineral and nutrient quality. During times of prolonged drought stress this process is hindered and such compounds cannot be properly allocated through the plant creating a dormant physiological status preventing plant growth. This relationship is especially critical in desert areas where precipitation values are generally low. Therefore, drought can represent a major economic burden to cattle producers, with animal performance being altered due to low nutrient availability (Scasta et al., 2015).

Stresses experienced in utero have been reported to impact fetal growth and development through a process referred to as fetal programming (Barker et al., 1993). It has been well documented that maternal nutrient intake during gestation can alter progeny calf health and performance (Corah et al., 1975; Martin et al., 2007; Funston et al., 2010). Decreased dam nutrient intake can also influence female offspring puberty attainment and pregnancy rates (Sasser et

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al., 1988; Selk et al., 1988). Although the influences of nutrient intake in gestating range cattle have been well documented, little is known regarding the direct effects of precipitation on fetal growth and programming. The objective of the study was to determine the effect of precipitation level during specific time points during gestation on beef progeny performance.

MATERIALS AND METHODS

Precipitation data and cattle performance data on 2,429 cows were collected from 1969 through 2015 at the NMSU Chihuahuan Desert Rangeland Research Center (CDRRC; Las Cruces, NM). All animal procedures and facilities were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Cow Data

Cow performance data obtained from the CDRRC included cow weaning BW, yearling BW, and corresponding calf's weaning BW, yearling BW, adjusted 205 d BW and calf sex. Cows were stratified by year (n = 2429), calf data were grouped and averaged together by year. Female progeny data evaluated age at time of first calf, productive years in the herd, number of total calves, and calving after 8 yr of age. Calf weaning BW, yearling BW, and adjusted 205 d BW were analyzed.

Precipitation Data

Precipitation data were gathered and compiled from 25 standard rain gauges located in subdivided pastures. Precipitation averages were calculated for each month and parameters set to analyze a time frame for the first trimester of gestation, which coincides with the monsoon season (July-September), late gestation (December-February), and total duration from conception to gestation (April-April). For each time period, a z-value was given based on the overall precipitation mean creating three classes of treatments defined as low (**Low**; z-value ≤ -1.00), average (**Avg**; z-value -0.99 - + 0.99), and high (**High**; z-value ≥ +1.00) using the similar method reported by Palmer (1965). Standard deviation (σ) being determined based on the overall precipitation mean from the 46 yr period utilizing the following formula.

$$\sigma=\sqrt{\sigma^2}$$

A z-value was determined using the formula below, for each designated period. Each period mean was calculated based on the calf's birth year.

$$Z = (X - \mu) / \sigma$$

Z represents the z-value and X is the value of the element in this cse it is the period mean. Additionally, μ represents population mean which is the overall period precipitation mean.

Statistical Analysis

Data were analyzed using the PROC MIXED and GLMMIX procedure of SAS (SAS Inst. Inc., Cary, NY). For reproduction and growth performance, cow was considered the experimental unit with precipitation treatment and year as the fixed effects. Calf birth BW, weaning BW, and adjusted 205 d BW means were separated using the LSMEANS procedure of SAS and P-values ≤ 0.05 were considered significant and tendencies were considered at a $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Calf Weights and Performance Data

Calf growth performance is reported in Table 1. Calves experiencing low precipitation throughout gestation had reduced ($P \leq 0.05$) birth and weaning BW compared to calves exposed to high precipitation levels in utero. Previous research conducted by Gaston et al. (2016), investigating dry and wet conditions on beef cattle performance revealed similar BW differences from cool season dry and wet classes of calves entering the feedlot with normal and wet classes being heavier. Similarly, calves had increased (P = 0.04) weaning BW and adjusted 205 d weaning BW (P = 0.03) if precipitation levels were high during the monsoon period when compared to the low treatment group. These results contradict Long et al. (2010) where 20 crossbred heifers were allotted to one of two diets, low 55% of NRC requirements or moderate 100% of NRC requirements at d 32 of gestation until d 83. They reported no differences in calf birth BW or postnatal growth, suggesting that perhaps the short duration of nutrient deprivation was not great enough to elicit an effect. Meyer et al. (2016) reported calves in Iowa classified as being born following wet year had greater feedlot entry BW as well as reduced number of days on feed. Interestingly, they reported a decrease in 12-th rib fat and marbling scores for calves in the high compared with low precipitation class.

Droughts role on forage yield, results in decreased plant quality (Oelberg, 1956), which consequently effects diet quality for grazing livestock. Cattle consuming poor quality or reduced nutrient diets during different periods of gestation may result in unfavorable restrictions on progeny growth and development

TABLE 1. Brangus calf growth performance based on precipitation received in-utero

	Treatments[1]				
Item	Low	Avg	High	SEM	*P*-value
Birth Weight (kg)					
Total[2]	31[a]	35[b]	37[b]	1.6	0.05
Monsoon[3]	32	35	35	1.7	0.29
Late Gest.[4]	34	35	-	0.9	0.89
Weaning Weight (kg)					
Total	218[a]	238[ab]	258[b]	15.8	0.05
Monsoon	218[a]	236[ab]	259[b]	14.4	0.04
Late Gest	227	244	-	7.6	0.12
Adj 205d Weight (kg)					
Total	210	252	247	47.7	0.56
Monsoon	211[a]	230[ab]	249[b]	13.2	0.03
Late Gest.	221	236	-	7.0	0.13

[a,b]Within a row means with different subscripts are different ($P < 0.05$).

[1]Treatments are Low (Low) = z-value ≤ -0.99, Average (Avg) = z-value -0.99 to +0.99 of the mean, and High (High) = z-value ≥ 0.99.

[2]Total = Summation of monthly average rainfall from average conception date to average parturition date.

[3]Monsoon= Summation of monthly average rainfall received the first trimester from July to September.

[4]Late Gest= Summation of monthly average rainfall received during the last trimester Dec-Mar.

TABLE 2. Brangus female progeny performance based on precipitation received in-utero

	Treatments[1]				
Item	Low	Avg	High	SEM	*P*-value
Age at first calving					
Total[2]	2.22	2.26	2.20	0.09	0.82
Monsoon[3]	2.27	2.20	2.33	0.10	0.17
Late Gest.[4]	2.24	2.25	-	0.05	0.90
Calved at 2 yr of age,%					
Total	82	85	86	5	0.77
Monsoon	77	87	81	5	0.06
Late Gest.	85	84	-	2	0.81
Calved after 8 yr,%					
Total	38	15	16	5	<0.0001
Monsoon	48	18	9	5	<0.0001
Late Gest.	18	19	-	3	0.66
Number of calves					
Total	5.23[a]	3.52[bc]	3.88[c]	0.36	<0.0001
Monsoon	5.90[a]	3.78[bc]	3.11[c]	0.40	<0.0001
Late Gest.	3.63	3.95	-	0.19	0.22

[a–c]Within a row means with different subscripts are different (P <0.05).

[1]Treatments are Low (Low) = z-value ≤ -0.99, Average (Avg) = z-value -0.99 to +0.99 of the mean, and High (High) = z-value ≥ 0.99.

[2]Total = Summation of monthly average rainfall from average conception date to average parturition date.

[3]Monsoon= Summation of monthly average rainfall received the first trimester from July to September.

[4]Late Gest= Summation of monthly average rainfall received during the last trimester Dec-Mar.

(Wu et al., 2006; Long et al., 2009; Ford et al., 2007). Forage availability is one of the most limiting factors for producers grazing cattle in a semi-arid environment. However, desert plants have evolved to withstand prolonged drought by becoming more efficient in utilization of resources, preserving nutrient content. Pnueli et al. (2002) investigated the physiological mechanism of plant dormancy in a desert legume as a defense against such harsh environmental conditions. DNA was extracted from the plant revealing a combination of avoidance and resistance strategies as the plant decreased protein denaturation and up regulated transcription factors for a pathogenesis-related protein. This suggests that native desert plant species have the ability to withstand harsh drought conditions with decreasing nutrient content prior to drought exposure. This mechanism may explain, in part, why there were no differences in birth BW, weaning BW, and adjusted 205 d BW among treatments during the late gestation period.

Female Reproductive Performance Data

Female progeny reproductive performance is reported in Table 2. Though there were no differences ($P \geq 0.17$) between treatment groups for age at first calf, females exposed to low precipitation levels in utero produced a greater ($P < 0.0001$) number of calves compared with animals experiencing average or high treatments throughout gestation. Additionally, there tended to be a greater ($P = 0.06$) proportion of heifers experiencing average precipitation during early gestation (monsoon) calving by 2 yr of age when compared to their counterparts. Funston et al. (2010) reported dams in late gestation grazing winter range or corn residue, with or without a protein supplementation had similar ($P = 0.97$) pregnancy rates in female offspring. Low treatment females from the same periods also had a greater percentage calve after 8 yr of age ($P < 0.0001$). This phenomenon may suggest that below average precipitation calves in-utero are genetically adapted to the intended environment.

Drought has affected cattle production for years, which, from a management perspective, causes selection pressure for more efficient cattle to offset economic deficits which may contribute to increased reproductive efficiency in the herd (Adams et al., 1996; Scasta et al., 2015). These results suggest that precipitation does play a role on progeny performance and may have an influence as early as conception in programming cow productivity and longevity.

Implications

Results from this study suggest precipitation level during gestation can elicit a programming like effect on progeny growth and reproductive performance. Utilizing precipitation values offers producers the potential for selecting efficient females specific to the herd's environment. Further research is warranted investigating precipitation levels experienced during early to mid-gestation and the effects on progeny performance and efficiency.

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Retrospective analyses of birthdate and growth in beef heifers categorized by puberty and pregnancy status

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ABSTRACT: Crossbred, Angus-based heifer records from 2002 to 2015 (n=1,404 observations) were used to retrospectively evaluate if birthdate and BW measured from weaning to final pregnancy diagnosis differed when heifers were categorized by 5 different approaches: 1) pubertal status prior to estrous synchronization, 2) whether or not detected in estrus and AI, 3) heifers impregnated by AI vs all others, 4) final pregnancy status, and 5) a 5-way classification accounting for AI and pregnancy status (AI pregnant, heifers subjected to AI that subsequently conceived to bull, heifers not AI that were impregnated by bull, heifers subjected to AI that were not pregnant, heifers not AI and not pregnant). Six BW measures were included in the analysis: weaning, mid-winter, pre-synchronization, AI, first pregnancy diagnosis, and final pregnancy diagnosis. From BW, 8 ADG measures were calculated. Measures available for most year also included Julian birthdate (n=749 observations) and pubertal status prior to synchronization (n=1,204 observations). The GLIMMIX procedure of SAS was used to retrospectively evaluate if Julian birthdate, cycling status prior to breeding, and BW measures collected from weaning through final pregnancy diagnosis varied among these categories. The general outcome from the different analyses was that the more favorable reproductive outcome within each categorization approach was associated with heifers being born earlier and achieving heavier BW at weaning and all subsequent BW. An exception to this generalization was BW up to the first pregnancy diagnosis did not differ ($P > 0.10$) for heifers that conceived to AI and those that conceived to natural service. Heifers that were AI but did not become pregnant tended ($P \leq 0.10$) to be heavier than heifers not inseminated and did not become pregnant. Collectively, the results support the concept that earlier birth in the calving season and greater preweaning growth (reflected by heavier BW at weaning and subsequent weights) are associated with desirable reproductive response in replacement beef heifers.

Key words: beef heifer, birthdate, growth, pregnancy, puberty
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INTRODUCTION

Numerous studies have reported inverse correlations between postweaning growth rate and age at puberty and pregnancy rates in heifers (reviewed by Funston et al., 2012). Vraspir et al. (2013) concluded pregnancy rate was greater for heifers achieving puberty prior to breeding, which was influenced by age and BW. An increasing body of literature has also demonstrated postbreeding management can have significant impacts on breeding success (Perry, 2016). However, limited information exists on which time points prior to or after the breeding season have the greatest impacts on reproductive success.

Therefore the objective of this study was to retrospectively analyze heifer data to evaluate how growth from weaning up to and through the breeding season differed when beef heifers were categorized by puberty and pregnancy status.

MATERIALS AND METHODS

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

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Crossbred, Angus-based heifers were purchased and arrived at the West Central Research and Extension Center (**WCREC**), North Platte, NE, at or shortly after weaning. Various development treatments (Harris et al., 2008; Funston and Larson, 2011; Summers et al., 2014) were applied overwinter. Prior to estrus synchronization, 2 blood samples were collected 10 d apart via caudal venipuncture to determine pubertal status. Heifers with greater than 1 ng/mL progesterone at either collection were considered pubertal. Heifers were synchronized using the melengestrol acetate-prostaglandin $F_{2\alpha}$ (**MGA-PG**) protocol. Heifers received MGA for 14 d. On d 33, PG was injected i.m. Heat detection followed for 5 d after injection. Heifers were observed for standing estrus and AI 12 h later. Heifers not expressing estrus were not inseminated. Ten days after last AI clean-up bulls were added at a 1:50 bull to heifer ratio for a 60 d breeding season. Pregnancy diagnosis was conducted via transrectal ultrasonography 45 d following AI and again 45 d after bull removal.

Records from heifers born in 2002 to 2015 (n=1,404) were analyzed. Birthdate was available for a subset of heifers (n=749) and included in the analysis. Pubertal status prior to estrus synchronization was available for all but 2 year. Six BW measures were recorded for most heifers: weaning, mid-winter, pre-synchronization, AI, first pregnancy diagnosis, and final pregnancy diagnosis. Weaning BW was either a single measure or an average of 2 measures taken within 2 to 3 wk after arriving at WCREC and occurred from mid-October to early November. Mid-winter BW was measured between mid-January to mid-February. Pre-synchronization was averaged from 2 BW taken 10 d apart immediately prior to MGA supplementation and occurred in mid-April. Body weight recorded at AI was measured at PG injection in late May. First pregnancy diagnosis BW occurred in mid-July, approximately 45 d after the last AI date. Final pregnancy diagnosis BW was measured in late September, approximately 45 d after bull removal. From the BW measures, 8 ADG measures were calculated for the database: weaning to mid-winter, mid-winter to pre-synchronization, pre-synchronization to AI, AI to first pregnancy diagnosis, first pregnancy diagnosis to final pregnancy diagnosis, weaning to pre-synchronization, weaning to AI, and AI to final pregnancy diagnosis.

Heifer were categorized by 5 different approaches: 1) pubertal status prior to estrus synchronization, 2) whether or not detected in estrus and inseminated, 3) heifers impregnated by AI vs all others, 4) final pregnancy status (yes vs no), and 5) a 5-way classification accounting for AI and pregnancy status. The 5-way classification included heifers conceiving to AI (**AIpreg**, n=816), heifers subjected to AI that subsequently conceived to bull (**AIbull**, n=351), heifers not inseminated that were impregnated by bull (**notAIpreg**, n=150), heifers inseminated that were not pregnant (**AIopen**, n=93), heifers not inseminated and not pregnant (**notAIopen**, n=28).

TABLE 1. Comparison of BW and ADG between cyclic vs. non-cyclic heifers prior to estrus synchronization. Heifers were synchronized with a melengestrol acetate (MGA)-PG protocol.

	Non-cyclic	Cyclic	SE	*P*-value
Julian birthdate	83	80	1.5	0.04
BW, kg				
Weaning[1]	231	239	1.6	< 0.01
Mid winter[2]	272	283	2.1	< 0.01
Pre-synchronization[3]	316	338	2.4	< 0.01
AI[4]	344	366	2.4	< 0.01
First pregnancy diagnosis[5]	366	380	2.3	< 0.01
Final pregnancy diagnosis[6]	419	433	2.4	< 0.01
ADG, kg/d				
Weaning to mid winter	0.45	0.50	0.01	< 0.01
Mid winter to pre-synchronization	0.66	0.72	0.02	< 0.01
Pre-synchronization to AI	0.76	0.72	0.02	0.06
AI to first pregnancy diagnosis	0.46	0.36	0.02	< 0.01
First to final pregnancy diagnosis	0.75	0.72	0.01	0.08
Weaning to pre-synchronization	0.49	0.58	0.01	< 0.01
Weaning to AI	0.54	0.60	0.01	< 0.01
AI to final pregnancy diagnosis	0.52	0.47	0.01	< 0.01

[1]Mid October to early November.

[2]Mid January to mid February.

[3]Average of 2 BW measured 10 d apart immediately prior to MGA supplementation.

[4]Late May, measured at PG injection.

[5]Mid July, approximately 45 d after last AI d.

[6]Late September, approximately 45 d after bull removal from 60-d breeding season.

The GLIMMIX procedure of SAS was used to retrospectively evaluate if Julian birthdate, cycling status prior to breeding, and BW measures collected from weaning through final pregnancy diagnosis varied among the categories in the different approaches. The model included birth year as a random effect and fixed effect of pubertal status/breeding/pregnancy category.

RESULTS AND DISCUSSION

Pubertal Status Prior to Estrus Synchronization

Pubertal heifers prior to estrus synchronization were born 3 d earlier ($P = 0.04$; 83 vs 80 Julian birthdate, non-pubertal vs pubertal, respectively; Table 1). Pubertal heifers were heavier ($P < 0.01$) at all BW measured. In addition, pubertal heifers gained more ($P < 0.01$) BW from weaning to mid-winter, mid-winter to pre-synchronization, and consequently weaning to pre-synchronization. While pubertal heifers also exhibited greater ($P < 0.01$) ADG from weaning to AI,

TABLE 2. Comparison of BW and ADG between AI and non-AI heifers. Heifers were synchronized with a melengestrol acetate (MGA)-PG protocol and only heifers displaying estrus behavior were inseminated.

	Not AI	AI	SE	*P*-value
Julian birthdate	85	81	2.0	0.08
BW, kg				
Weaning[1]	231	235	1.9	0.04
Mid winter[2]	273	279	2.4	0.03
Pre-synchronization[3]	322	329	2.9	0.02
AI[4]	349	356	3.0	0.01
First pregnancy diagnosis[5]	370	376	2.6	0.03
Final pregnancy diagnosis[6]	420	429	2.8	< 0.01
ADG, kg/d				
Weaning to mid winter	0.47	0.48	0.01	0.37
Mid winter to pre-synchronization	0.68	0.70	0.02	0.44
Pre- synchronization to AI	0.70	0.73	0.02	0.17
AI to first pregnancy diagnosis	0.47	0.45	0.02	0.25
First to final pregnancy diagnosis	0.68	0.73	0.02	< 0.01
Weaning to pre-synchronization	0.54	0.55	0.01	0.24
Weaning to AI	0.56	0.58	0.01	0.11
AI to final pregnancy diagnosis	0.51	0.52	0.01	0.19

[1]Mid October to early November.

[2]Mid January to mid February.

[3]Average of 2 BW measured 10 d apart immediately prior to MGA supplementation.

[4]Late May, measured at PG injection.

[5]Mid July, approximately 45 d after last AI d.

[6]Late September, approximately 45 d after bull removal from 60-d breeding season.

non-pubertal heifers tended to gain more ($P = 0.06$) from pre-synchronization to AI (0.76 vs 0.72 kg/d, non-pubertal vs pubertal, respectively).

Heifers not cycling prior to estrus synchronization did gain more ($P < 0.01$) from AI to first pregnancy diagnosis and AI to final pregnancy diagnosis. This pattern of gain, where non- pubertal heifers have increased ADG during the breeding season indicates these heifers were possibly later maturing, with greater mature BW or exhibiting a compensatory gain due to better quality forage available during synchronization and breeding periods.

Estrus Detection and Artificial Insemination

Heifers observed in estrus and inseminated tended to be older ($P = 0.08$, 85 vs 81 Julian birthdate, non-pubertal vs pubertal, respectively; Table 2). Inseminated heifers were heavier ($P \leq 0.04$) at weaning and all subsequent BW compared with heifers not inseminated.

Gains were similar between categories, except from first to final pregnancy diagnosis where inseminated heifers had greater ADG ($P < 0.01$, 0.68 vs 0.73 kg/d, non-pubertal vs pubertal, respectively).

TABLE 3. Comparison of BW and ADG between heifers pregnant by AI vs heifers pregnant by natural service or open.

	Not AI pregnant	AI pregnant	SE	*P*-value
Julian birthdate	83	80	1.4	0.02
BW, kg				
Weaning[1]	233	235	1.3	0.10
Mid winter[2]	276	279	1.7	0.16
Pre-synchronization[3]	327	329	2.0	0.36
AI[4]	354	356	2.0	0.37
First pregnancy diagnosis[5]	374	376	1.8	0.23
Final pregnancy diagnosis[6]	424	431	1.9	< 0.01
ADG, kg/d				
Weaning to mid winter	0.48	0.48	0.01	0.75
Mid winter to pre-synchronization	0.71	0.68	0.01	0.04
Pre-synchronization to AI	0.73	0.73	0.01	0.83
AI to first pregnancy diagnosis	0.44	0.46	0.02	0.39
First to final pregnancy diagnosis	0.68	0.75	0.01	< 0.01
Weaning to pre-synchronization	0.55	0.55	0.01	0.85
Weaning to AI	0.58	0.58	0.01	0.87
AI to final pregnancy diagnosis	0.49	0.53	0.01	< 0.01

[1]Mid October to early November.

[2]Mid January to mid February.

[3]Average of 2 BW measured 10 d apart immediately prior to MGA supplementation.

[4]Late May, measured at PG injection.

[5]Mid July, approximately 45 d after last AI d.

[6]Late September, approximately 45 d after bull removal from 60-d breeding season.

AI Pregnancy vs All Others

Heifers pregnant by AI were born 3 d earlier ($P = 0.02$, 80 vs 83 Julian birthdate, AI pregnant vs not AI pregnant, respectively; Table 3) than their counterparts. Body weight was similar between the 2 categories until final pregnancy diagnosis, where heifers not pregnant by AI weighed less ($P < 0.01$, 424 vs 431 kg, not AI pregnant vs AI pregnant, respectively). This may be due to the difference in weight of the pregnancy.

Heifers not pregnant by AI did gain more from mid-winter to pre-synchronization ($P = 0.04$, 0.71 vs 0.68 kg/d, non-pubertal vs pubertal, respectively); however, they gained less ($P < 0.01$) BW from first to final pregnancy diagnosis and AI to final pregnancy diagnosis. Again the greater gains for AI pregnant heifer may be due to the weight of the actual pregnancy.

Final Pregnancy Status

Although age was similar between nonpregnant and pregnant heifers ($P = 0.15$, Table 4), BW was greater ($P < 0.01$) for pregnant heifers (AI and bull-bred) at all measures.

TABLE 4. Comparison of BW and ADG between nonpregnant vs pregnant (includes AI and natural service) heifers.

	Not Pregnant	Pregnant	SE	*P*-value
Julian birthdate	85	81	2.3	0.15
BW, kg				
Weaning[1]	227	235	2.2	< 0.01
Mid winter[2]	271	278	3.0	0.01
Pre-synchronization[3]	318	329	3.4	< 0.01
AI[4]	346	356	3.4	0.01
First pregnancy diagnosis[5]	365	376	3.2	< 0.01
Final pregnancy diagnosis[6]	413	429	3.2	< 0.01
ADG, kg/d				
Weaning to mid winter	0.48	0.48	0.02	0.74
Mid winter to pre-synchronization	0.65	0.70	0.02	0.06
Pre-synchronization to AI	0.74	0.73	0.03	0.69
AI to first pregnancy diagnosis	0.41	0.45	0.03	0.11
First to final pregnancy diagnosis	0.67	0.73	0.02	< 0.01
Weaning to pre-synchronization	0.53	0.55	0.01	0.19
Weaning to AI	0.57	0.58	0.01	0.25
AI to final pregnancy diagnosis	0.48	0.52	0.01	< 0.01

[1]Mid October to early November.

[2]Mid January to mid February.

[3]Average of 2 BW measured 10 d apart immediately prior to MGA supplementation.

[4]Late May, measured at PG injection.

[5]Mid July, approximately 45 d after last AI d.

[6]Late September, approximately 45 d after bull removal from 60-d breeding season.

Nonpregnant heifers tended ($P = 0.06$) to gain less from mid-winter to pre-synchronization (0.65 vs 0.70 kg/d, nonpregnant vs pregnant, respectively). Nonpregnant heifers also gained less ($P < 0.01$) from first to final pregnancy diagnosis and AI to final pregnancy diagnosis. This is also demonstrated in Figure 1 shows this is true with animals grouped by AI status.

5-way Classification of AI and Pregnancy Status

Julian date of birth did not differ due to AI and pregnancy classification, although the numeric trend was for AIpreg to be born earlier. The percentage of heifers cycling prior to estrus synchronization differed among the groupings, following the pattern of being greatest in AIpreg (76%), intermediate in AIopen (62%), and least in notAIopen (24%). Percentage cycling in heifers bred by bulls (70% for AIbull and notAIpreg) was similar to AIpreg and AIopen (76% and 62%, AIpreg and AIopen, respectively). Measures of weaning BW differed due to classification, and these differences persisted through the remaining measurements (Figure 1). The general pattern was for heifers in the AIpreg and AIbull groups to be heavier than AIopen, which tended

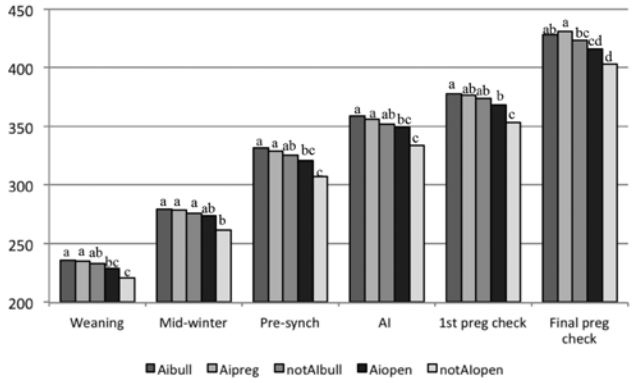

Figure 1. Retrospective comparison of BW at 6 different time points among heifers inseminated but became pregnant by natural service (AIbull), heifers pregnant by AI (AIpreg), heifers not inseminated but became pregnant by natural service (notAIpreg), inseminated heifers not becoming pregnant (AIopen), and heifers not inseminated and not becoming pregnant (notAIopen). Bars with different letters differ ($P < 0.05$). AIopen tended ($P < 0.1$) to differ from notAIopen.

or were heavier than notAIopen. Heifers in the non-AIpreg group were intermediate, but not statistically different between the AIpreg, AIbull, and AIopen.

Implications

Birthdate and weaning BW seem to be the 2 major factors accounting for whether heifers became pregnant or not, as the differences in BW between pregnant and not pregnant heifers remained similar through the breeding season. A greater percentage of heifers becoming pregnant were also cyclical prior to estrus synchronization compared with nonpregnant heifers. When selecting replacement heifers, producers should consider heifer age and BW.

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Absorption and elimination of [^{14}C]-dicoumarol in goats[1]

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ABSTRACT: Dicoumarol is a well-known toxic anti-coagulant which contributes to sweet clover poisoning in exposed cattle. Although dicoumarol was used in the 1950s as a human blood thinner, almost no data are available describing dicoumarol absorption, distribution, metabolism and excretion [ADME] in animals used for food. To our knowledge, ADME studies of dicoumarol have never been conducted in ruminant animals, which are the natural targets of dicoumarol poisoning. To this end, three healthy castrated male goats (31 ± 7 kg) were orally administered a single bolus dose of 5 ± 0.02 mg/kg of [^{14}C]-dicoumarol, while one goat was used as a control. Blood, urine and feces were collected for a 72-h period after dosing. At 72 h, goats were euthanized and adipose tissue, bile, blood, bone, brain, gastrointestinal contents, gastrointestinal tissue, heart, lungs, liver, kidneys, pancreas, skeletal muscle, skin, spleen, and thyroid were collected for the analysis of total radioactive residues. The cumulative excretion of radioactive residues in urine represented 18.4 ± 2.4% of the total dose by 72 h. Peak levels of radioactive residues occurred at 12-18 h in urine; at 72 h, urinary radioactivity represented 2.4 ± 0.9 μg/g of dicoumarol equivalents. Blood levels of total radioactive residues peaked at 5.1 ± 2 μg/g (C_{max}) of dicoumarol equivalents at 5.97 ± 1.27 h (t_{max}). The half-life of radioactive residue absorption was 1.81 ± 0.75 h; the half-life of elimination from blood was 14.16 ± 3.0 h. At 72 h radioactivity in blood remained at 0.22 ± 0.04 μg/g dicoumarol equivalents. Evidence for the biliary elimination of dicoumarol (and/or its metabolites) was present with 72 h bile containing 1.56 ± 0.29 μg/g of dicoumarol equivalents. These preliminary results indicate that dicoumarol is rapidly absorbed, and slowly eliminated in goats.

Key words: dicoumarol, goat, sweet clover, toxin
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INTRODUCTION

Dicoumarol is a naturally occurring coumarin derivative that was formerly used as a therapeutic anticoagulant in humans, until replaced by its semi-synthetic analogue warfarin (Weiner et al., 1950; Link, 1959). Dicoumarol is a fungal metabolite of coumarin that forms in moldy hay or silage containing sweet clover and is biosynthesized by fungi from the *Penicillium* genus. Once ingested and absorbed, dicoumarol inhibits vitamin K induced blood-clotting factors (Link, 1959; Pritchard et al., 1983). This naturally occurring anticoagulant is responsible for sweet clover poisoning in cattle (Campbell et al., 1941; Link, 1959; Wignall et al., 1961; Puschner et al., 1998). Cattle that feed on moldy sweet clover hay may develop internal hemorrhaging and may die after high dose dicoumarol exposure (Quick, 1937; Smith and Brink, 1938; Pritchard et al., 1983; Dwyer et al., 2003). Although the toxin was developed as an anticoagulant for human and animal therapy (Clark et al., 2000), very little is known about its metabolic fate and disposition in ruminant animals, especially in the context of animal toxicity. Furthermore, the kinetics of dicoumarol residue depletion from milk and/or meat products after food animal exposures remains uninvestigated. Due to limited availability of data on dicoumarol absorption, distribution, metabolism, and excretion (ADME) in ruminants, this study was conducted with the objective of characterizing the fate of radioactive residues after oral administration of [^{14}C]-dicoumarol to healthy wether goats.

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MATERIALS AND METHODS

Chemicals

Formaldehyde, 4-hydroxycoumarin, and HPLC grade water and methanol were purchased from Sigma Aldrich (St. Louis, MO). [^{14}C]-Formaldehyde (55 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

Synthesis and Characterization of [^{14}C]-Dicoumarol

[^{14}C]-Dicoumarol with a radiochemical purity of 98.7% was synthesized in-house according to the method described by Lee et al. (1950) to a specific activity of 529 dpm/µg (0.08 mCi/mmol). The chemical purity of dicoumarol was >98% as assessed by proton and carbon NMR and LC-MS/MS detection.

In a 500 mL round-bottom flask 2 g of 4-hydroxycoumarin was mixed with 200 mL of HPLC grade water and stirred at room temperature for 5 min. To this mixture 1 mL of non-radiolabeled formaldehyde was added along with 1 mCi of [^{14}C]-formaldehyde. The reaction mixture was stirred at room temperature for 72 h. Upon completion (confirmed by thin layer chromatography) the reaction mixture was filtered and washed with cold water. The product was dried under vacuum at 60 ºC and a total yield of 1.998 g (96%; melting point, 289.5 – 290.5 ºC) was obtained.

The specific activity of the [^{14}C]-dicoumarol was determined chromatographically. Non-radiolabeled dicoumarol (10 mg) was weighed, dissolved in acetone (170 µL of 1M NaOH and 20 µL of formic acid were used to increase solubility), and transferred to a 5 mL volumetric flask and used as stock solution. Five dilutions (250 µg/mL, 500 µg/mL, 1000 µg/mL, 1500 µg/mL and 2000 µg/mL) were prepared and aliquots of each were injected into the chromatographic system, the respective peak areas were recorded and a standard curve was created. Subsequently, aliquots of radiolabeled dicoumarol were injected in triplicate, peak areas were recorded, and [^{14}C]-dicoumarol peaks were trapped into liquid scintillation counting (LSC) vials as dicoumarol eluted from the chromatography column. Samples were counted for a minimum of 10 min using a liquid scintillation counter. The specific activity was determined by dividing the radioactivity (dpm) of each trapped peak by its mass (µg) as determined by regression of its peak area on the dicoumarol standard curve.

Test Animals, Dosing, and Sample Collection

A detailed animal use protocol was approved by the RRVARC BRL IACUC prior to the purchase of animals. Four wether goats (31 ± 7 kg) were purchased and delivered to the Biosciences Research Lab where they were allowed to adapt for 25 d prior to start of study. Animals were housed indoors with access to a mixed alfalfa-grass hay, a grain supplement, and water. Before dosing, goats were trained to eat and reside in metabolism crates.

On study d 0, goats were placed in individual metabolism crates and were dosed orally, via a balling gun, with a gelatin capsule containing [^{14}C]-dicoumarol (5 ± 0.02 mg/kg BW). The control goat (used as a source of blank blood, excreta, and tissues) did not receive dicoumarol. Blood samples (3 mL) were collected in heparinized vacutainer tubes via 21-gauge needles at 0, 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 8, 12, 18, 24, 30, 36, 48, 60 and 72 h. Urine and feces were collected in increments corresponding to 0-4, 4-8, 8-12, 12-18, 18-24, 24-30, 30-36, 36-48, 48-60, and 60-72 h relative to dosing. At collection, total weights of the urine and feces were recorded and excreta was placed in prelabeled containers. All samples were subsequently stored at -20 °C or less. At 72 h after dosing, goats were euthanized by captive bolt stunning followed by immediate exsanguination. Adipose tissue, bile, blood, bone, brain, gastrointestinal contents, gastrointestinal tissue, heart, lungs, liver, kidneys, mammary glands, pancreas, remainder of the carcass, rumen contents, rumen tissue, skeletal muscle, skin, spleen, and thyroid glands were collected, weighed, and frozen.

Determination of Total Radioactive Residues

For each sample set, control (blank) matrix of urine, bile (4 aliquots each) and blood (5 aliquots) were weighed in scintillation vials, mixed with LSC fluid and background radioactivity determined (10 min counts). The mean of control replicates of a sample was considered as the background radioactivity. For every sample set, a limit of detection (LOD) was calculated using the average of background dpm plus three standard deviations (SDs) of the average. When samples from dosed animals exhibited average dpm values lower than the LOD, they were considered to have undetectable radioactivity.

Bile. Samples were thawed at room temperature, vortexed, and quadruplicate 50 µL aliquots were transferred to liquid scintillation vials (7 mL). Aliquot weights were recorded and 6 mL of LSC fluid was added and mixed. Each vial was counted for 10 min (PerkinElmer Tri-carb 2019 TR).

Urine. Radioactivity in urine was determined as described for bile except that radioactivity in quadruplicate 775 µL aliquots was quantified.

Blood. Triplicate blood samples (250 µL) were weighed into combustion cups and capped with combus-

TABLE 1. Urinary elimination of radioactive (^{14}C-dicou-marol equivalents; percent of dose) over a 72 h period after a single oral dose (5 ± 0.02 mg/kg BW)

	Radiocarbon (% of dose)		
Time (h)	Goat 146	Goat 147	Goat 148
0 – 4	1.47	-[1]	1.23
4 – 8	2.28	1.46	3.24
8 – 12	1.55	4.89	2.64
12 - 18	6.76	2.90	3.34
18 - 24	0.08*	1.58	2.22
24 - 30	2.60	1.32	1.57
30 - 36	2.08	1.27	1.33
36 - 48	1.24	1.38	1.06
48 - 60	2.29	1.02	0.75
60 - 72	0.65	0.57	0.38

[1]Container was dry, water rinse of container residues.

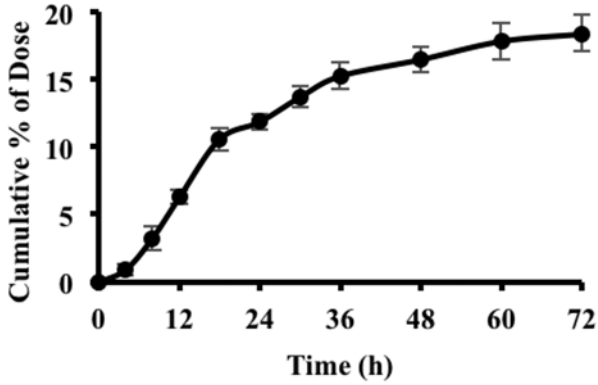

Figure 1. Cumulative elimination of urinary radiocarbon (percent of dose, mean ± SE) as a function of time. Goats (n = 3) were provided a single oral dose (5 ± 0.02 mg/kg BW) of ^{14}C-dicoumarol at 0 h.

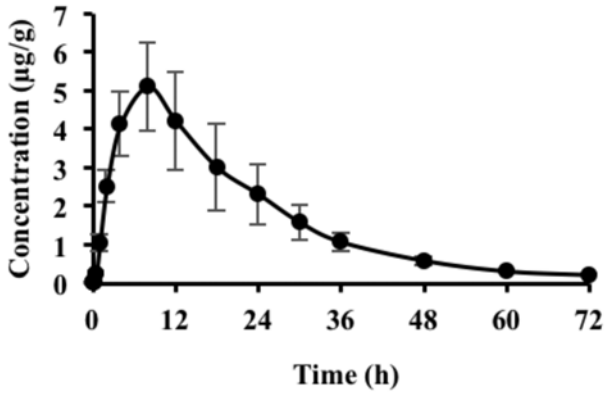

Figure 2. The concentration (µg/g, mean ± SE) of total radioactive residues (^{14}C-dicoumarol equivalents) in whole blood after administration of a single oral dose (5 ± 0.02 mg/kg BW) of ^{14}C-dicoumarol to goats.

tion pads. Blood samples were oxidized on a PerkinElmer Model 307 sample oxidizer and $^{14}CO_2$ from each sample was trapped into 8 mL of Carbo-Sorb E (Perkin-Elmer, Waltham, MA), mixed with Permafluor E (+) LSC fluid, and each sample counted for 10 min.

Kinetic Parameter Estimates. Absorption and elimination parameters of total radioactive residues, expressed as [^{14}C]-dicoumarol equivalents, were estimated for individual goats using whole blood data and non-compartmental pharmacokinetic modeling methods (PK-Solution software; Summit Research Services, Montrose, CO). Because this was an observational study, statistical comparisons were not appropriate.

RESULTS AND DISCUSSION

A total of 6.7 ± 1.6 µCi of radiocarbon was excreted into urine during the 72 h study period, representing 17.89 ± 1.56 % of the total oral dose. Table 1, which shows the urinary elimination of radiocarbon by time of collection, clearly indicates that by 4 h after dosing radiocarbon had started to appear in urine, suggesting rapid absorption. Concentrations of urinary radioactivity peaked between 8-18 h. For goats 146 and 148, radioactive material excreted in urine between 12-18 h were 6.76 and 3.34 % of the total dose, respectively. Similarly, 4.89 % of the dosed radioactivity was present in urine collected at 8-12 h in goat 147. Goats were still excreting radioactivity during the 60-72 h collection period with urine containing 0.65, 0.57 and 0.38 % of the total dose for goats 146, 147 and 148, respectively. Cumulative elimination of radiocarbon in urine is shown in Figure 1. The highest measured concentrations of [^{14}C]-dicoumarol equivalents in blood were observed at 8 h after a single dose oral administration of 5 ± 0.02 mg/kg BW (Figure 2). The average calculated values for C_{max}, t_{max}, absorption half-life and elimination half-life obtained from the three animals were 5.10 ± 2.03 µg/g, 5.97 ± 1.27 h, 1.81 ± 0.75 h, 14.16 ± 3 h, respectively (Table 2). Radioactive residues in bile were at much higher concentrations (1.56 ± 0.29 µg/g) than in blood (0.22 ± 0.04 µg/g) at 72 h in all three animals. These data suggest that the hepatic drug metabolism and subsequent biliary excretion of dicoumarol could be significant in goats. Husain et al. (1973) noted that biliary excretion was the major route of dicoumarol elimination in rats; by 4 h after dosing, approximately eight times more radioactive residue was excreted in bile (~8% of the dose) than in urine. In goats, the concentration of radioactive material in urine after 72 h was numerically higher (2.4 ± 0.9 µg/g) than in bile (1.56 ± 0.29 µg/g). Data reported from a human study, suggests that elimination of dicoumarol by kidneys is negligible (< 1%) and the rate of absorption and transformation is very slow and variable (Weiner et al., 1950). In addition, the same study reported that after a single dose, dicoumarol was

TABLE 2. Kinetic parameter estimates of total radioactive residues in whole blood (C_{max}, t_{max}, absorption half-life and elimination half-life) of goats

	Pharmacokinetic Parameters			
Goat no.	C_{max} (μg/g)	t_{max} (h)	Absorption Half-Life (h)	Elimination Half-Life (h)
146	7.30	7.40	2.64	12.14
147	3.30	5.00	1.19	17.61
148	4.70	5.50	1.60	12.73
Avg[1]	5.10	5.97	1.81	14.16
SD	2.03	1.27	0.75	3.00

[1]Avg = average.

retained within the body for a week or more due to protein binding in blood and other tissues.

These preliminary data suggest that after a single, non-toxic exposure dicoumarol and/or its metabolites are easily detected after 72 h and that urinary elimination accounted for less than 20% of the total dose. The role of fecal elimination in goats is being determined, but if similar to humans and dogs (Weiner et al., 1950) could be minimal in ruminants. Total radioactive residues analysis of skeletal muscles and other organs, and identification of metabolites in excreta and tissues will provide insights into possible toxic mechanisms of dicoumarol in ruminants. Further, identification of radioactive residues in edible tissues may provide insights into possible food-safety issues. Planned studies investigating ADME of [^{14}C]-dicoumarol in lactating animals will provide insights with respect to possible dicoumarol exposures to pre-weaned cattle and to perhaps human exposures through commercial milk.

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Response of pregnant ewes to ovalbumin inoculation, antiovalbumin antibody transfer to lambs, and temporal changes in antiovalbumin antibody[1,2]

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ABSTRACT: Factors that affect the decay of maternally-derived IgG and the ability of neonatal lambs to produce protective amounts of their own IgG are not well understood. Three experiments were conducted to evaluate colostral transfer of antiovalbumin IgG (OV-IgG) from ewes to lambs and responses of young lambs to ovalbumin inoculation. In Exp. 1, pregnant ewes (n = 10/group) were inoculated with either ovalbumin or control (saline) suspensions at ≈ 42 (primary) and ≈ 14 (secondary) d before lambing. In Exp. 2, lambs (n = 20/group) born to and nursing ewes that had been immunized against ovalbumin were inoculated with either ovalbumin or control suspensions at age 1 (primary) and 15 (secondary) d. In Exp. 3, lambs (n = 20/group) born to ewes that were naive to ovalbumin received 1 of 2 inoculation types, control or ovalbumin suspension, according to 1 of 2 inoculation schedules, at age 1 and 15 d or 28 and 42 d. According to original inoculation type, lambs also received a booster inoculation at age ≈ 159 d. In Exp. 1, ovalbumin inoculation increased ($P < 0.001$) ewe serum OV-IgG. Serum OV-IgG was greater ($P < 0.0001$) throughout the sampling period in lambs born to and nursing ovalbumin-treated ewes than in lambs from control ewes. In Exp. 2, ovalbumin inoculation of lambs at age 1 d reduced ($P < 0.04$) maternally-derived serum OV-IgG through age 15 d compared with saline inoculation. However, after age 15 d, serum OV-IgG was greater ($P < 0.001$) in ovalbumin-treated than in control lambs. In Exp. 3, in lambs that received ovalbumin inoculations at age 1 and 15 d, serum OV-IgG increased through age 21 d but declined after age 28 d ($P < 0.004$). In lambs that received ovalbumin inoculations at age 28 and 42 d, OV-IgG increased ($P < 0.001$) steadily through age 21 d and stabilized thereafter. In lambs that received ovalbumin inoculation at age 28 and 42 d, serum OV-IgG in blood samples collected just before the booster was greater ($P < 0.006$) than observed in lambs in the other 3 treatment groups. Inoculating lambs within 24 h after birth may reduce circulating maternally derived antibodies, but it can induce an immune response. The results of this study support vaccinating ewes against common pathogens during late pregnancy to ensure that lambs receive adequate colostrum soon after birth.

Key words: antibody, passive transfer, sheep, vaccination

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INTRODUCTION

Lamb wellbeing largely depends on the transfer of antibodies, particularly IgG, via colostrum, from ewes to newborn lambs (Hunter et al., 1977; Nowak and Poindron, 2006; Massimini et al., 2006). This passive transfer of IgG is critical because lambs are born with almost no IgG of their own (Halliday, 1971; Sawyer et al., 1977). Indeed, the risk of lamb mortality decreased as serum IgG concentrations in neonatal lambs increased (Berggren-Thomas et al., 1987; Gilbert et al., 1988; Christley et al., 2003). Moreover, newborn lambs do not have a fully competent immune system and cannot produce adequate amounts of IgG for several weeks after birth (Sawyer et al., 1977; Tizard,

[1]Authors wish to thank the technical staff at the U.S. Sheep Experiment Station for assisting with the experiments.

[2]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). The USDA is an equal opportunity provider and employer.

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1996; de la Rosa et al., 1997; Nowak and Poindron, 2006). Perhaps the single most effective method for enhancing lamb serum IgG concentrations is to immunize late-pregnant ewes against common pathogens, such as the various Clostridium species (ASI, 2015).

Maternally-derived IgG only provide short-term immunity to common pathogens. This is because IgG decays over time to such a degree that sufficient IgG is lacking to effectively control the growth of disease-causing organisms (Tizard, 1996; Nowak and Poindron, 2006). Although the production, transfer, and uptake of maternal IgG in lambs are fairly well understood, factors that affect the decay of maternally-derived IgG and the ability of lambs to produce protective amounts of their own IgG are not well understood. Thus, we conducted 3 experiments to quantify the 1) response of pregnant ewes to ovalbumin inoculation 2) antiovalbumin IgG (**OV-IgG**) transfer to lambs from colostrum, 3) changes over time in OV-IgG in lambs, and 4) response of young lambs to ovalbumin inoculation.

MATERIALS AND METHODS

Animal husbandry, treatment delivery, and sampling

All animal-related procedures were reviewed and approved by an Institutional Animal Care and Use Committee. Except for injections, sampling, and time of turn out on pasture, study ewes and lambs were managed according to standard U.S. Sheep Experiment Station procedures (see Leeds et al., 2012). In all experiments, lambing was spontaneous, rather than induced, and ewes lambed outdoors. Within approximately 30 min after parturition, each ewe and her lamb(s) were moved indoors into individual bonding pens (i.e., lambing jugs). Experienced personnel monitored all newborn lambs and confirmed that each lamb consumed colostrum from its dam, but the volume consumed was not quantified.

In all experiments, either ewes, lambs, or both received inoculations of either an ovalbumin suspension [12 mg of ovalbumin (>90% pure; Sigma-Aldrich, St. Louis, MO), 1 mL of aluminum hydroxide gel adjuvant (Alhydrogel; Accurate Chemical and Scientific Corp., Westbury, NY), and 1 mL of sterile isotonic saline] or a saline (control) suspension (1 mL of Alhydrogel and 1 mL of sterile isotonic saline). All injections were subcutaneous on the neck of each animal. Needles were changed between animals. Syringes used for ovalbumin were not used for control injections, and vice versa.

For Exp. 1 and 2, colostrum was stripped from the udder of each ewe, and a 10-mL sample was collected from each ewe and stored at −20° C until OV-IgG was quantified. In all experiments, blood samples were collected from a jugular vein (Vacutainer SST Plus Blood Collection Tubes (BD, Franklin Lakes, NJ). Samples were allowed to clot at 4° C and stored for a minimum of 2 h at 4° C. Serum was collected after centrifugation at 1,781 × g and 4° C. Samples were stored at −20° C until OV-IgG concentrations were quantified.

Experimental design and management

In Exp. 1, treatments were prepartum inoculations of ewes with ovalbumin (n = 10) or control (n = 10) suspensions. Pregnant white-faced ewes were assigned to treatment and inoculated accordingly at ≈ 42 (primary) and ≈ 14 (secondary) d before lambing. Blood samples (10 mL) were collected from each ewe at 7-d intervals beginning immediately after the primary inoculation through ≈ 35 d after lambing. On the day after lambing, an additional 10-mL blood sample was collected from each ewe, and a 5-mL blood sample was collected from each lamb. Additional 5-mL blood samples were collected from each lamb at weekly intervals through 35 d of age.

In Exp. 2, treatments were inoculation of neonatal lambs with ovalbumin (n = 20) or control (n = 20) suspensions. Initially, pregnant white-faced ewes (n = 40) were inoculated against ovalbumin ≈ 42 and ≈ 21 d prepartum, respectively. Ewes that were part of Exp. 1 were not used for Exp. 2. After ewes lambed, neonatal lambs were assigned to treatment and inoculated accordingly at d 1 (primary) and d 15 (secondary) of age. Blood samples (15 mL) were collected just before each injection and at weekly intervals until the lambs were ≈ 36 d of age.

In Exp. 3, treatments were inoculation type (control vs. ovalbumin suspensions) and inoculation schedule (d 1 and d 15 of age vs. d 28 and d 42 of age), arranged in a 2 × 2 factorial array, applied to neonatal lambs (n = 20/treatment group). The treatment groups were: 1) inoculation with control suspension on d 1 and repeated on d 15; 2) inoculation with ovalbumin suspension on d 1 and repeated on d 15; 3) inoculation with control suspension on d 28 and repeated on d 42; 4) inoculation with ovalbumin suspension on d 28 and repeated on d 42. Treatments were randomized in blocks and assigned to neonatal white-faced ewe lambs right after they were born. In addition, at an average of 159 d of age, which was soon after weaning, lambs received inoculations of either control or ovalbumin suspensions (i.e., booster) that was consistent with their original treatment inoculation type. To prevent the transfer of maternal antiovalbumin IgG to lambs, ewes that produced lambs for this experiment had not been immunized against ovalbumin or used in Exp. 1 or 2. However, blood samples were collected from the

ewes and analyzed to determine whether these restrictions had been met. Blood samples (15 mL) were collected from each lamb before the d-1 and d-15 and before the d-28 and d-42 inoculations. Additional 15-mL blood samples were collected weekly for 5 wk. Blood samples (15 mL) were also collected immediately before the postweaning inoculations and at weekly intervals for the next 4 wk.

Measurement of Antiovalbumin IgG

For all experiments, OV-IgG in serum and colostrum were quantified using an ELISA as described previously (Mousel et al., 2008), with the exception that data are reported as optical density units (odu), indicative of concentration, as opposed to titers. Colostrum samples were centrifuged multiple times to remove fat.

Statistical Analyses

The Proc GLM and Mixed procedures in SAS (SAS Inst. Inc., Cary, NC) were used to analyze endpoint and repeated data, respectively. For repeated measures in Exp. 1 and 2, the models included terms for treatment, day of sampling, and the treatment × day interaction. Day was classified as repeated, and animal within treatment was the subject. For Exp. 3, period of treatment (early in life vs. after weaning) was confounded with age and initial treatments, and the experiment was not designed to avoid this confounding. Thus, the data were sorted and analyzed within period to gain a clear understanding of treatment effects early in life and then after weaning. Models for repeated measures in Exp. 3 included terms for injection schedule, injection type, day of sampling, and all interactions. Day was classified as repeated, and animal within injection schedule × injection type was the subject. For endpoint measures (e.g., colostrum IgG), similar models were used with the exception that day and day interactions were removed. Least-squares means and pooled SE were reported. Proc GLM, using models comparable to those used in Proc Mixed, were used to generate the estimates of variance that were used to calculate pooled SE. Alpha level was set as ≤ 0.05.

RESULTS

For saline (control)-inoculated ewes and lambs in Exp. 1 and 2, the results of the serum OV-IgG ELISA indicated an apparent OV-IgG concentration (i.e., odu) in serum. However, we considered this to be primarily the effects of nonspecific binding and not actual OV-IgG. Saline-inoculated animals did not receive ovalbumin, and thus could not have developed IgG against ovalbumin.

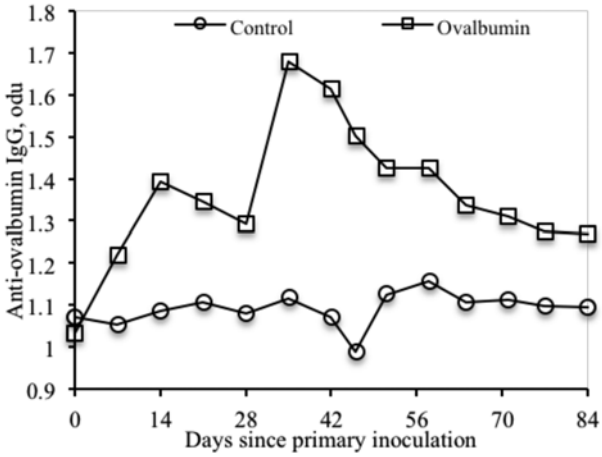

Figure 1. Serum antiovalbumin IgG concentrations (odu; optical density units) in periparturient ewes inoculated subcutaneously with either ovalbumin (squares) or control saline (circles) suspensions. Inoculations were given at approximately 42 (primary) and 14 (secondary) d before lambing to 10 pregnant ewes in each group. The X-axis scale is adjusted to days since inoculation. Therefore, the primary inoculation (i.e., ≈ 42 d before lambing) is set as d 0 of inoculation, while the secondary inoculation is set at d 28; lambing occurred near d 42 on the scale. Ovalbumin inoculation increased ($P < 0.001$) serum antiovalbumin IgG compared with saline-inoculated ewes. The apparent positive response in serum of the control group is nonspecific binding (i.e., background noise) inherent to the ELISA assay and not antiovalbumin IgG. Values are least-squares means, with a pooled SE of 0.007 odu.

Experiment 1: Antiovalbumin IgG production in ewes and passive transfer to lambs

In the ewes, ovalbumin inoculation increased ($P < 0.001$) serum OV-IgG (control, 1.06 odu, vs. ovalbumin treated, 1.34 odu; pooled SE = 0.007 odu), and serum OV-IgG changed with time after primary inoculation (ovalbumin treatment × day interaction, $P < 0.001$; Fig. 1). Also, ovalbumin inoculation increased ($P < 0.001$) colostrum OV-IgG (control, 0.98 odu, vs. ovalbumin treated, 1.47 odu; pooled SE = 0.008 odu).

In the lambs, serum OV-IgG was greater ($P < 0.0001$) in lambs from ovalbumin-treated ewes than in lambs from control ewes (1.48 odu vs. 0.69 odu; pooled SE = 0.024 odu). Antiovalbumin IgG in lambs from ovalbumin-treated ewes was greater ($P < 0.001$) than in control lambs throughout the sampling period, and day and the treatment × day interaction were not significant ($P = 0.34$ and 0.64, respectively).

Experiment 2: Antiovalbumin IgG after ovalbumin inoculation into neonatal lambs

In the ewes, serum OV-IgG averaged 1.14 odu (pooled SE = 0.017 odu). Antibodies increased ($P < 0.0001$) over time after the primary inoculation (d 0, 0.82 odu; d 21, 1.21 odu; and d 45, 1.37, odu). Colostrum OV-IgG averaged 1.38 odu (pooled SE = 0.022 odu).

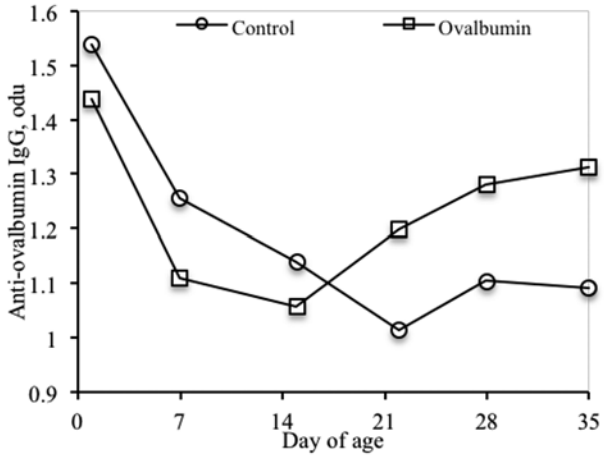

Figure 2. Serum antiovalbumin IgG concentrations (odu; optical density units) in neonatal lambs inoculated subcutaneously with ovalbumin (squares) or control saline (circles) suspensions. Inoculations were given at 1 (primary) and 15 (secondary) d of age to 20 lambs in each group. Lambs in both treatment groups were born to and nursed ewes that had been inoculated with ovalbumin during late pregnancy. Antiovalbumin IgG was less ($P < 0.04$) in ovalbumin-treated than in control lambs from d 1 to d 15, whereas after d 15 antiovalbumin IgG was greater ($P < 0.001$) in ovalbumin-treated than in control lambs. Values are least-squares means, with a pooled SE of 0.009 odu.

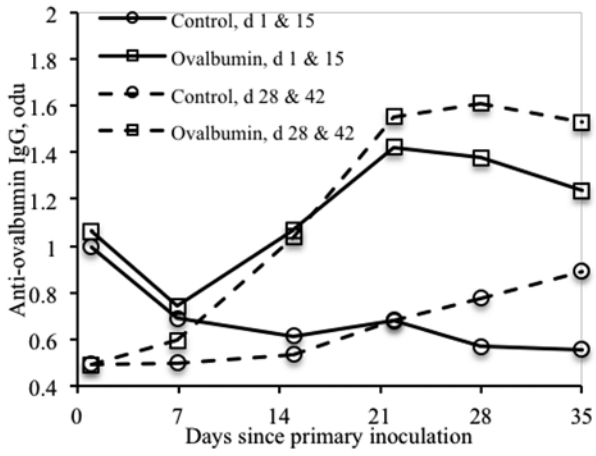

Figure 3. Serum antiovalbumin IgG concentrations (odu; optical density units) in lambs (n = 20/group) that were inoculated subcutaneously with either ovalbumin (squares) or control saline (circles) suspensions. Inoculation schedules for primary and secondary inoculations, respectively, were either at d 1 and 15 of age (solid lines) or d 28 and 42 of age (dashed lines). Injection type (saline vs. ovalbumin; $P < 0.0001$), but not injection schedule (d 1 and d 15 vs. d 28 and d 42 of age; $P = 0.59$), affected antiovalbumin IgG. The injection type × injection schedule interaction was not significant ($P = 0.84$). Time after injection affected ($P < 0.0001$) antiovalbumin IgG, and the injection type × time and injection schedule × time interactions were significant ($P < 0.0001$). The injection type × injection schedule × time interaction was not significant ($P = 0.34$). Values are least-squares means, with a pooled SE of 0.01 odu.

In the lambs, the main effect of ovalbumin treatnt did not affect serum OV-IgG (control, 1.19 odu, vs. ovalbumin, 1.23 odu; pooled SE = 0.009 odu). However, the treatment × day-of-age interaction was significant ($P < 0.0001$). From d 1 to d 15, OV-IgG was less ($P < 0.04$) in ovalbumin-treated than in control lambs (Fig. 2). After d 15, OV-IgG was greater ($P < 0.001$) in ovalbumin-treated than in control lambs (Fig. 2).

Experiment 3: Antibody response of lambs inoculated in an early period of life and again in a period after weaning

During the early period of life, inoculation type (control, 0.67 odu, vs. ovalbumin, 1.14 odu; $P < 0.0001$), but not schedule (d 1 and d 15, 0.92 odu, vs. d 28 and d 42 of age, 0.89 odu; $P = 0.59$), affected OV-IgG (pooled SE = 0.01 odu), and the inoculation type × schedule interaction was not significant ($P = 0.84$). Antiovalbumin IgG changed ($P < 0.0001$) with time after inoculation (Fig. 3), and the inoculation type × time and schedule × time interactions were significant ($P < 0.0001$). The inoculation type × schedule × time interaction was not significant ($P = 0.34$).

In lambs that received ovalbumin inoculation on d 1 and d 15 of age, serum OV-IgG increased ($P < 0.001$) from d 7 to d 21 after ovalbumin treatment, but OV-IgG decreased ($P < 0.004$) after d 28 (Fig. 3). In lambs that received ovalbumin inoculations on d 28 and d 42 of age, OV-IgG increased ($P < 0.001$) steadily until d 21 after treatment and then stabilized after d 21 (Fig. 3). Apparent OV-IgG in lambs that received saline inoculations on d 1 and d 15 or on d 28 and d 42 of age changed over time, but after d 7 of the sampling period they remained less ($P < 0.005$) than OV-IgG in ovalbumin-treated lambs (Fig. 3).

During the period after weaning, inoculation type (control, 0.93 odu, vs. ovalbumin, 1.38 odu; $P < 0.0001$), but not initial inoculation schedule (d 1 and d 15, 1.12 odu, vs. d 28 and d 42 of age, 1.19 odu; $P = 0.59$), affected serum OV-IgG (pooled SE = 0.013 odu), and the inoculation type × schedule interaction was not significant ($P = 0.08$). Antiovalbumin IgG changed ($P < 0.0001$) with time after inoculation (Fig. 3), and the inoculation type × time interaction was significant ($P < 0.0001$). The inoculation schedule × time and inoculation type × inoculation schedule × time interactions were not significant ($P = 0.86$ and 0.68, respectively).

Serum OV-IgG in lambs that had received ovalbumin inoculation early in life (i.e., d 1 and d 15 or d 28 and d 42 of age) increased ($P \leq 0.003$) after a booster inoculation of ovalbumin on approximately d 159 of age (Fig. 4). In lambs that received ovalbumin inoculation on d 28 and d 42 of life, average OV-IgG in blood samples collected just before the d-159 booster was greater ($P < 0.006$) than observed in lambs in the other 3 treatment groups (Fig. 4). Apparent serum OV-IgG in lambs that received control inoculation remained less than 1.0 odu throughout the sampling period (Fig. 4).

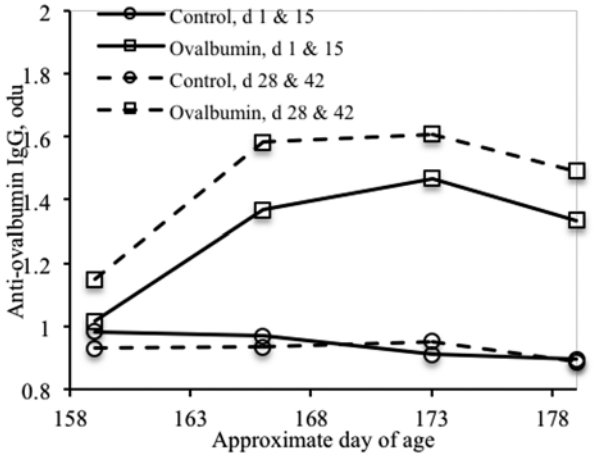

Figure 4. Serum antiovalbumin IgG concentrations (odu; optical density units) in lambs (n = 20/group) that were inoculated subcutaneously with either ovalbumin (squares) or control saline (circles) suspensions. Inoculation schedules for primary and secondary inoculations, respectively, were either at d 1 and 15 of age (solid lines) or d 28 and 42 of age (dashed lines). At an average of age 159 d, lambs received an additional inoculation (i.e., booster) respective to their original inoculation type. Injection type (control vs. ovalbumin; $P < 0.0001$), but not initial injection schedule (d 1 and d 15 vs. d 28 and d 42 of age; $P = 0.59$), affected antiovalbumin IgG. The injection type × injection schedule interaction was not significant ($P = 0.08$). Time after injection ($P < 0.0001$) affected antiovalbumin IgG, and the injection type × time interaction was significant ($P < 0.0001$). Values are least-squares means, with a pooled SE of 0.013 odu.

DISCUSSION

In this study, inoculating ewes with ovalbumin during the last approximately 6 wk of pregnancy increased OV-IgG in blood serum and colostrum. Antiovalbumin IgG was apparently transferred to lambs because OV-IgG was greater in lambs born to ovalbumin-treated ewes than in lambs born to control ewes. These effects of ovalbumin inoculation during late pregnancy and the relationship between colostrum IgG and passive immunity in lambs are consistent with published data (Hunter et al., 1977; Sawyer et al., 1977). Moreover, these responses underlie the recommendations to vaccinate ewes against common pathogens, such as *Clostridium perfringens* and *C. tetani*, during late pregnancy and to ensure that lambs receive adequate colostrum soon after birth (ASI, 2015).

The changes over time in OV-IgG in lambs were evaluated in Exp. 1 and 2. In Exp. 1, serum OV-IgG in lambs born to ovalbumin-treated ewes was increased on d 1, compared with lambs born to control ewes, and OV-IgG remained increased and fairly stable throughout the 6-wk sampling period. However, in Exp. 2, OV-IgG in control lambs, also born to ovalbumin-treated ewes, decreased, compared with d-1 OV-IgG, during the first 3 wk of life and then remained decreased throughout the sampling period. The changes over time in Exp. 2 are consistent with the expected decay of passive immunity (Tizard, 1996) during the first few weeks of life and with changes in concentrations of antibodies to ε-toxin of *C. perfringens* type D in lambs (de la Rosa et al., 1997). By contrast, the lack of substantial changes over time in Exp. 1 is not consistent with previous data, and we have no plausible explanation for this apparent maintenance of passive immunity to ovalbumin.

Experiments 2 and 3 were conducted to address questions surrounding the response of young lambs to immunization. In Exp. 2, ovalbumin inoculation of lambs on d 1 of life reduced, compared with control treatment, OV-IgG; lambs in this experiment were from ewes that had been inoculated against ovalbumin. We had anticipated a reduction in OV-IgG after ovalbumin inoculation because IgG of maternal origin can neutralize vaccine antigens, and this can reduce antibody titers (Tizard, 1996; Chappuis, 1998; Roitt et al., 1998; Premenko-Lanier et al., 2006; Demirjian and Levy, 2009).

In Exp. 3, ovalbumin inoculation of lambs, which were from ewes not inoculated against ovalbumin, on d 1 and d 15 or on d 28 and d 42 of life increased OV-IgG, as did ovalbumin inoculation of lambs on d 15 in Exp. 2. The inoculation schedule (i.e., d 1 and d 15 vs. d 28 and d 42) had little overall effect on OV-IgG during the immediate 35-d sampling period. Based on previous publications, we had expected ovalbumin inoculation to induce some production of OV-IgG. Even though newborns may not have a fully competent immune system, they can produce antibodies (Tizard, 1996; Chappuis, 1998). The lingering questions about sheep, and other ruminants, focus on whether neonates can produce protective amounts of antibodies and whether inoculating neonates against common pathogens reduces passive immunity enough to make them more susceptible to the pathogens. The literature does not provide clear answers to these questions, but there is clear evidence that inoculating young lambs (i.e., d 1 and d 21 or d 42 of age) against *C. perfringens* did not induce a significant immune response or improve survival rates and feedlot performance (Hoefler and Hallford, 1985; de la Rosa et al., 1997).

In Exp. 3, ovalbumin inoculation on approximately d 159 of age increased OV-IgG in lambs that had been inoculated with ovalbumin on d 1 and d 15 or on d 28 and d 42 of life. However, the experimental design did not allow us to determine whether the d-159 injection acted as a booster or as a primary inoculation. Nevertheless, the effects of a d-159 inoculation are consistent with the recommendation to inoculate lambs at the time of weaning (ASI, 2015).

Implications

Maternal antibodies that were produced in response to inoculations during late pregnancy were

transferred to lambs via colostrum, and this validated a critical element of the experimental model. Inoculating young lambs may reduce circulating maternally derived antibodies, but it can induce an immune response in the lambs. Overall, the results of this study support the recommendations to vaccinate ewes against common pathogens during late pregnancy and to ensure that lambs receive adequate colostrum soon after birth. The results of this study do not support the notion of inoculating newborn (e.g., < 24-h old) lambs, which may or may not be able to produce an adequate immune response, instead of inoculating late pregnant ewes and gaining the colostrum-mediated advantage of passive immunity against common pathogens.

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Variation of forage yield and quality within different forage groups for western Canada[1]

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ABSTRACT: A consecutive 4-yr random block design field experiment with four replicates for each harvest treatment was conducted at Agriculture and Agri-Food Canada-Swift Current Research and Development Centre. The objective of this study was to determine the variations in dry matter yield and forage quality as defined by acid detergent fiber % (ADF), neutral detergent fiber % (NDF), crude protein % (CP), and organic matter digestibility % (OMD) within three forage groups that consisted of C3, C4 grasses and legumes. All the forages were harvested at the same time in the spring, summer and fall. The results indicated that: C3 Northern wheatgrass (*Agropyron dasystachyum*) and Needle and thread grass (*Hesperostipa comata*) had relatively high productions in spring compared to the other grasses, while C4 Prairie sandreed (*Calamovilfa longifolia*) had relatively high productions at any season. When comprehensively considering which forages could provide high forage yields and quality potential and optimal harvest dates across different growth stages for the semiarid prairie region, the following results were observed: within the C3 grass group, Meadow bromegrass (*Bromus riparius*) and Hybrid bromegrass (*B. inermis Leyss.*×*Bromus riparius*) show promise to be used for summer and fall; within the legumes group, Canadian milkvetch (*Astragalus canadensis*) potentially could be used at any season. This information may be useful for forage breeders and producers to select optimum harvest times for each species and to decide which forage mixture could potentially be suitable to provide good forage production and quality.

Key words: C3, C4, grasses, harvest season, legumes
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INTRODUCTION

Forage quality and production have a direct impact on livestock performance on pasture and it is necessary to understand what characteristics change forage yield and quality to optimize productivity (Ball et al., 2001). However, both quantity and quality of forage varies tremendously among and within different types of forages (Kirkman and de Faccio Carvalho, 2003). Understanding what affects forage yield and quality for specific plants can allow producers to better select forage species that can complement each other to improve animal production and reduce nutrient supplementation requirements. This information can also provide opportunities for forage breeders to consider optimizing forage mixtures and research directions.

In semiarid mixed-grasslands, the major forage types used in ruminant production are commonly segmented into two categories: legumes and grasses. Grasses are further classified into C3 and C4 grasses in term of the ecological significance linked with the type of photosynthesis and leaf structure (McKendry, 2002; Mischkolz et al., 2013). Different photosynthetic and metabolic pathways associated with C3 and C4 plants affect forage production and chemical composition, digestibility and adaptability to changing environmental conditions. Generally, C3 type grasses have a lower photosynthetic potential and they

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cannot take advantage of the highest radiation portion (Ehleringer and Monson, 1993). They usually grow optimally at temperatures ranging from 20 to 25°C and generally growth slows when temperatures drop below 5°C (Baron and Bélanger, 2007). Grasses of C4 type have a more efficient photosynthetic system which allows them to exploit the high radiation. They usually have higher optimal growing temperatures, ranging from 30 to 35°C, and begin to grow when the soil temperature is above 15°C (Baron and Bélanger, 2007). Legumes are more efficient at gathering carbon dioxide and utilizing nitrogen from the atmosphere and recycled N in the soil. They usually have a faster photosynthesis rate than most of C3 and C4 grasses (Mischkolz et al., 2013).

Although forage quality has been defined in many ways, including palatability, intake, digestibility, nutrient content and other physical and/or chemical components (Newman et al., 2006), in the present paper, forage quality is defined as the sum total of acid detergent fiber % (ADF), neutral detergent fiber % (NDF), crude protein % (CP), and organic matter digestibility % (OMD) that influences ruminant's performance. There are many factors influencing forage quality and yield, such as the proportion of leaf to stem associated with plant composition and morphology, maturity stage at harvest connected with plant anatomy and physiology, forage species linked to genomics and genetics, and other climate and soil conditions (Haferkamp 1988; Ball et al., 2001; Barre et al., 2015). Nonetheless, the most important factors are forage species and harvest dates (Fulgueira et al., 2007).

The effects of forage species and harvest dates on forage yield and quality by grazing cattle have been evaluated (Beauchemin and Iwaasa, 1993; Popp et al., 1997), but comparisons within a forage group over the entire growing season, there is only limited information available. The objective of this study was to examine changes in dry matter yields and qualities (ADF, NDF, CP and OMD) among fifteen forage species that were categorized into three forage groups (C3, C4 and legumes), which were harvested in spring, summer and fall over a four-year period. Of specific interest was to identify potential yield and quality differences among the forages within the forage groups and harvest date that would be of interest to producers and forage breeders to extend the grazing season in the semiarid prairie region of Western Canada.

MATERIALS AND METHODS

Experimental Design and Data Collection

This research was conducted on Orthic Brown Chernozemic soil over a 4-yr period (2007-2010) at the Agriculture and Agri-Food Canada (AAFC) Swift Current Research and Development Centre (SCRDC) (lat. 50°25' N, long. 107°44' W, elev. 824 m), Saskatchewan, Canada. The experiment design was a randomized complete block with three harvest treatments and with four replications for each treatment. All perennial forage treatments were seeded at a rate of 98 PLS m^{-2} and a depth of 1.3 cm in plots of 6.0 m by 1.53 m in size. Each plot consisted of five seeded rows with a 30.5 cm row spacing. Species treatments were divided into three forage groups (C3 and C4 grasses and legumes). The C3 group included hybrid bromegrass (*Bromus inermis Leyss.* × *B. riparius*; HBG), meadow bromegrass (*B. riparius*; MBG), awned wheatgrass (*Agropyron subsecudum*; AWG), Canada wild rye (Elymus canadensis; CWR), western wheatgrass (*Pascopyrum smithii*; WWG), needle and thread grass (*Hesperostipa comata*; NT), northern wheatgrass (*A. dasystachyum*; NWG), green needlegrass (*Nassella viridula*; GNG), and June grass (*Koeleria macrantha*; JG). The C4 group included blue grama grass (*Bouteloua gracilis*; BG), little bluestem (*Schizachyrium scoparium*; LBS), and prairie sandreed (*Calamovilfa longifolia*; PSR). The legume group included creeping rooted alfalfa (*Medicago sativa*; CRA), Canadian milkvetch (*Astragalus canadensis*; CMV), and purple prairie clover (*Dalea purpurea*; PPC). Forage samples were clipped flushed to the ground on June 20, June 20, August 16 and October 10 in 2007, 2008, 2009 and 2010, respectively. Forage yield and quality were determined as described by Iwaasa et al. (2012). Due to poor establishment for PPC, CMV and LBS, not all replicates were available to be sampled or analyzed for all years.

Statistical Analysis Methods

We hypothesized that effects of year on forage yield and quality were random and similar among various forage species. Forage yield and quality data from each harvest date were tested for normality and homogeneity of variance, and then were subjected to analysis of variance (ANOVA) using R 3.3.2. Within each forage group, year and replication were treated as random effects, and forage species and harvest date were considered as main effects in the ANOVA. Lastly, to visually compare similarity and difference in yield and quality within different forage groups, multiple comparisons bar charts with standard error bars on dry matter yield, ADF, NDF, CP and OMD among forage species at different harvest dates were analyzed.

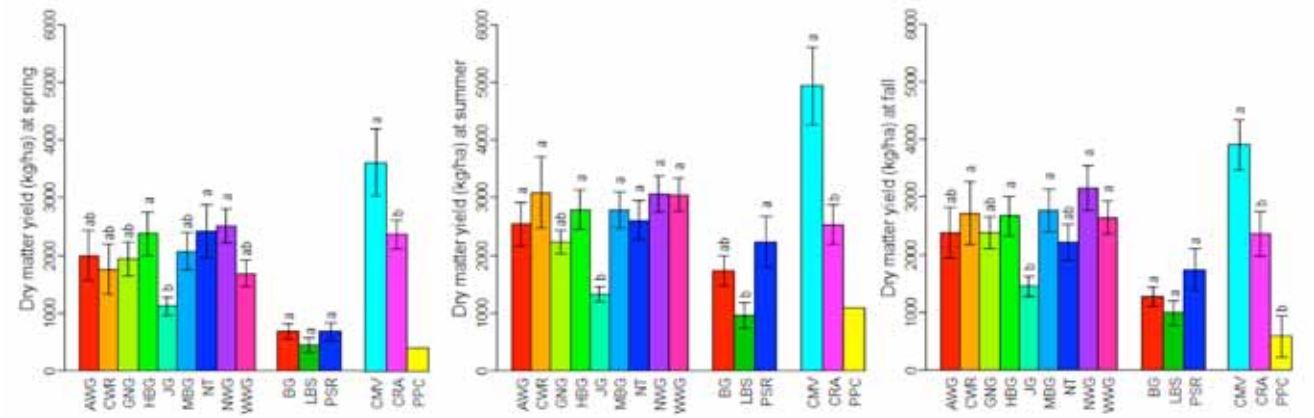

Figure 1. Changes in mean dry matter yield values (SE ± 5%) within C3, C4 and legume groups harvested at spring, summer, and fall at AAFC-SCRDC, respectively. Means across the figures with the same lettering are not significantly different ($P > 0.05$), and forage species without error bar and lettering denotes lacking of enough replicates.

RESULTS AND DISCUSSION

Changes of forage yield within three forage groups

As expected, forage yields for C3, C4 and legume groups generally increased with later harvest dates, and majority of them reached maximum-yield at summer. Forage yields in spring, summer and fall generally observed no differences ($P > 0.05$) among the C3 grasses except for JG. No significant differences were found among the C4 grasses except in summer and CMV was highest ($P < 0.05$) among the legumes regardless of season (Fig. 1).

Within the C3 group, HBG and NWG were generally in the top three numerically highest yielding species for the season and JG was the lowest yielding specie which was not unexpected. Especially in spring time the use of HBG might be better suited to be grazed than native grasses due to potentially higher forage yields and ecological reasons. Other C3 grasses like NT, CWR, MBG, and WWG were also relatively high yielding species at spring, summer and fall periods. Within the C4 group, PSR was the highest yielding specie in summer. Within the legume group, CMV was consistently the highest yielding specie over all the seasons.

Changes of forage quality within three forage groups

Decline in forage qualities (e.g., ADF and NDF percentages increased, and CP and OMD percentages decreased) were due to increase in cell wall and fiber levels with advancing physiological maturity (Buxton 1996; Jefferson et al. 2004). However, the rate of decline in forage quality within forage types can vary due to morphological and chemical compositional differences (Haferkamp 1988; Ball et al., 2001; Barre et al., 2015).

The ADF contents of NT and NWG were higher ($P < 0.05$) than the other C3 grasses in spring. In summer and fall, no differences ($P > 0.05$) in ADF values were generally observed among the C3 grasses except for WWG in summer. The PSR in ADF was higher ($P < 0.05$) than the other C4 grasses except in summer. The ADF content did not differ ($P > 0.05$) among legumes regardless of season. It is noteworthy, that in any season for WWG, the ADF values were always numerically lower than other C3 grasses. Also within the C3 group, JG, MBG and HBG were observed to have numerically lower ADF values in summer and fall. Within the C4 group, BG and LBS had lower ($P < 0.05$) ADF contents in spring and fall (Fig. 2).

The NDF content of NT, GNG and NWG were higher ($P < 0.05$) than the other C3 grasses in the spring and generally no differences ($P > 0.05$) were observed in summer and fall among the C3 grasses except for JG in the fall. The PSR in NDF was lower ($P < 0.05$) than the other C4 grasses at any season. No significant NDF differences ($P > 0.05$) were observed among legumes regardless of season. Within the C3 group, JG, HBG and MBG species were generally numerically lower ($P < 0.05$) in NDF at any season. Within the C4 group, LBS consistently was the lowest ($P < 0.05$) specie in NDF at any season (Fig. 2).

No differences ($P > 0.05$) in CP values were observed among the C3 and C4 grasses for any season. Only CMV was higher ($P < 0.05$) than other legumes in summer (Figure not shown).

The OMD values of CWR, HBG, JG, MBG, AWG and WWG were generally higher ($P < 0.05$) than that of other C3 grasses in spring; the OMD of JG was higher ($P < 0.05$) than the other C3 grasses in summer; while in the fall the OMD of JG and MBG were higher ($P < 0.05$) than the other C3 grasses. The OMD values did not differ ($P > 0.05$) among the C4 grasses at any season. The

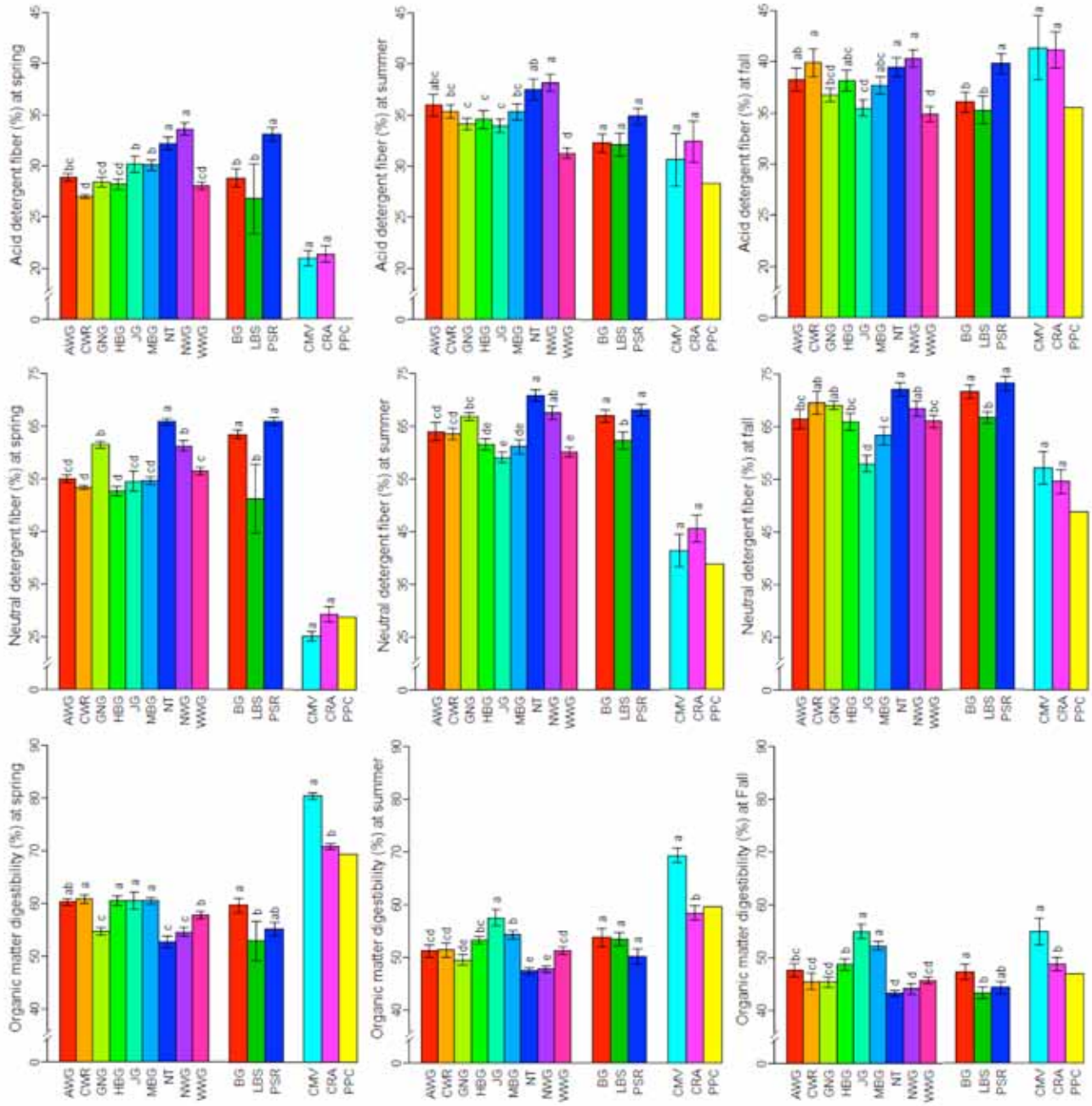

Figure 2. Changes in mean acid detergent fiber, neutral detergent fiber, and organic matter digestibility values (SE ± 5%) within C3, C4 and legume groups harvested at spring, summer, and fall at AAFC-SCRDC, respectively. Means across the figures with the same lettering are not significantly different ($P > 0.05$), and forage species without error bar and lettering denotes lacking of enough replicates.

OMD of CMV was the highest among legumes ($P < 0.05$) regardless of season. Within the C3 group, MBG and JG were generally numerically higher in digestibility regardless of season. Especially in summer and fall times the use of MBG and JG might be better suited to be grazed than native grasses due to better OMDs. Within the C4 group, BG was relatively the highest digestible specie at any season. Within the legumes group, CMV was consistently the highest digestible specie at any season (Fig. 2).

Based on all comparison results, within the C3 grasses, NWG was the highest productive specie and JG was the highest digestible specie at any season; and CWR was productive in the summer. In contrast, HBG and MBG forages were consistently higher in yielding and quality during the summer and fall. Within the C4 group, PSR was productive and BG had high forage quality at any season. Within the legumes group, CMV was relatively high in yield production and quality, especially in spring and summer. Generally, legumes produce higher forage quality than grasses. Grasses have relatively high yields but legumes have less fiber, higher crude protein and digestibility. Thus, we may get both higher yielding and forage quality mixtures by combining high yielding

species (e.g., NWG, HBG, MBG or LBS) from the C3 or C4 group and high forage quality species (e.g., CMV).

IMPLICATIONS

Summarizing the results, although effects of harvest seasons and forage species on yield and quality were not always statistically significant during different harvest dates, some interesting differences were observed. Comprehensively considering forage yield and quality at three harvest seasons, within the C3 group, both NWG and NT can be considered for use in spring, due to their high-yield; MBG and HBG can be used in summer and fall due to their relatively both high yields and qualities. Within C4 group, LBS could be utilized due to its relatively high quality at each season. Within legumes group, CMV could be recommended due to its relatively high yields and qualities at any season. This information may be useful for forage breeders and producers to select optimum harvest times for each species and decide which forage mixtures could potentially be suitable to provide good forage production and quality in the semiarid ecoregion of Western Canada.

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Effect of retained ownership and vertical integration within an integrated cropping system among yearling steers of differing frame score on feedlot performance, carcass measurements, and systems economics following delayed feedlot entry

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ABSTRACT: In a 3-year study, 288 yearling steers (n = 96 year^{-1}) of differing frame score (small frame SF: average 3.80; large frame LF: average 5.58) were used to evaluate retained ownership, vertical integration, extended grazing, and delayed feedlot entry. Steers were managed as a common group and backgrounded for modest gain (0.60 kg·day^{-1}) grazing unharvested corn supplemented with mixed hay and 0.37 kg·steer^{-1}·day^{-1} of a 32% CP supplement. The first week of May, the steers were assigned randomly to either feedlot control (FLOT) or grazing (GRAZ) treatments and then within treatment, the steers were stratified into SF and LF groups. The FLOT steers were delivered directly to the University of Wyoming, Sustainable Agriculture Research Extension Center (SAREC), Lingle, Wyoming, for growing and finishing and the GRAZ steers grazed native range (NR, 108 d), field pea-barley (32 d), and unharvested corn (71 d). Total FLOT days on feed (DOF) was 218 d whereas the GRAZ steers grazed perennial and annual forages for 211 d before transfer to the feedlot (82DOF). Small frame steers grew slower during grazing (P = 0.03) and feedlot finishing (P < 0.001) compared to the LF steers. Grazing cost and cost/kg of gain was less for the SF steers ($250.27 vs. $300.27/steer; $0.2525 vs. $0.2757/kg of gain). In the feedlot, LF steer starting BW (P < 0.001), end BW (P = 0.003), gain (P < 0.001), and ADG (P < 0.001) were greater. GRAZ steer compensatory gain in the feedlot, for the LF and SF steers, was 26.8 and 24.0% greater, respectively, compared to the LF and SF FLOT steers. Delaying feedlot entry reduced finishing cost of gain for the GRAZ system by an average 34.0% (P = 0.001). GRAZ steer HCW for LF and SF was greater than FLOT LF and SF steers (P = 0.01). Dressing percent (P < 0.001) and marbling score (P = 0.02) were greater for SF steers. LF steer REA (P = 0.001) was greater for both FLOT and GRAZ treatments. Percent Choice or better quality grade ranged from 91.7 to 97.2% across treatments, but did not differ (P = 0.11). Meat tenderness (P = 0.48) and cooking loss (P = 0.43) did not differ. SF steers were more profitable than LF steers at the end of grazing and both SF and LF GRAZ steers were more profitable than FLOT steers. Long-term extended grazing and reduced feedlot residency supported comparable meat quality and consistent profitability.

Key words: delayed feedlot entry, extended grazing, integrated crops and livestock, retained ownership, yearling steers

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INTRODUCTION

In the beef cattle business, profitability is impacted by a multitude of factors that are out of the producer's control. Therefore, producers are challenged with creating greater net value by retaining ownership using a vertically integrated system with the potential to increase beef value marketed. Harvested feeds increase slaughter breakeven cost (Anderson et al., 2005) compared to cattle managed extensively grazing for longer periods followed by an abbreviated concentrate feeding period (Lunt and Orme, 1987). Alternatively, in lieu

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of marketing calves directly after weaning, retaining ownership coupled with extended summer grazing allows producers to capitalize on compensatory growth (Lewis et al., 1990), reduced slaughter closeout cost (Shain et al., 2005), and greater integrated system net profit (Sindt et al., 1991). Yearling systems that utilize perennial pasture and grazing within a diverse, multi-crop, 5-year rotation enhance economically important muscle and marbling traits, when compared to a traditional feedlot growing and finishing program, and delaying feedlot entry has the greatest potential for system profitability (Senturklu, et al., 2014). Considering the results of Senturklu et al. (2014), the objective of this study was to evaluate small- and large frame yearling steers and compare a traditional feedlot system to a long-term extensive grazing system, with reduced feedlot residency, and document grazing and feedlot performance, carcass measurements, meat tenderness and cooking losses, and systems economics.

MATERIALS AND METHODS

The North Dakota State University institutional animal care and use committee (IACUC) approved all animal procedures.

Two hundred eighty-eight May-June born steer calves were weaned in November each year (n = 96/year; 2012, 2013, and 2014). Following a 7 d drylot weaning recovery period, the steers grazed unharvested corn, corn residue, and supplemental medium quality alfalfa-bromegrass (*Medicago sativa and Bromus inermis*) mixed hay plus 0.37 kg·steer^{-1}·day^{-1} of a 32% CP distiller's dried grain based supplement. The backgrounding gain goal was a modest 0.60 kg·steer^{-1}·day^{-1}.

The first week of May, the steers that averaged 12.0 months of age were randomly assigned based on weight, age, and frame score to either feedlot control (FLOT) or extended grazing (GRAZ) treatments and then stratified into SF and LF groups within treatment based on November hip height calculation (Beef Improvement Federation, 2010). Mean frame score values for FLOT were LF: 5.63 and SF: 3.82, and for GRAZ, the mean values were LF: 5.53 and SF 3.77. Each treatment consisted of three pen/pasture replicates of eight steers/replicate (n = 24). The FLOT steers were transferred directly to the University of Wyoming, Sustainable Agriculture Research Extension Center (SAREC), Lingle, Wyoming, for growing and finishing. The GRAZ steers grazed perennial native range (NR) pasture from the first week of May to mid-August (108 d). After NR grazing, the steers moved to annual forages grown in a 5-year, multi-crop, rotation consisting of spring wheat, 7-species cover crop, corn, field pea-barley, and sunflower. Crop use designation for the field pea-barley intercrop mix (*Pisum sativum, var. Arvika* and *Hordeum vulgare, var. Stockford*) and unharvested corn (*Zea mays*) was for grazing. Field pea-barley was grazed an average 32 d and unharvested corn 71 d. Annual forage grazing was considered complete when the higher quality corn aerial plant parts disappeared. After 211 d, GRAZ treatment steers were transferred to the University of Wyoming SAREC feedlot. In the feedlot, FLOT steer dietary starch concentration (corn) increased stepwise over 135 d, at which time the final finishing diet composition consisting of 5% alfalfa hay, 15% haylage, 80% corn, and a feedlot vitamin/mineral supplement was fed to the end of the study. By design, standing corn was the last crop grazed in the grazing sequence. This aided GRAZ steer stepwise transition to the same final finishing diet over an abbreviated period of 58 d. Based on ultrasound scan (Aloka SSD-500V; 3.5 MHz-17cm transducer and standoff) and order buyer confirmation, Cargill Meat Solutions, Ft. Morgan, Colorado, purchased the steers (Angus America grid).

Native range grazing cost determination was based on a constant cost per unit of body weight ($0.002579) and starting BW, end BW, and one-half of the total days grazed to arrive at an annual grazing cost, i.e. (0.002579 x start BW x (total days grazed/2) + (0.002579 x end BW x (total days grazed/2). Annual forage farming enterprise budgets were prepared using actual expenses for seed, fertilizer, chemical, inoculation, and crop insurance. These expenses were integrated with all other expenses was from the ND Farm and Ranch Business Management Education Program crop enterprise budgets (Region 4: 2013, 2014, and 2015).

Data was analyzed using Proc MIXED in SAS (SAS Institute Inc., Cary, NC. System treatment and year were fixed effects and pasture or pen was the experimental unit and random effect. Least-square means were utilized to identify levels of the effects and to control family-wise error adjusted with Tukey. Means were determined to be statistically significant using an alpha level of 0.05.

RESULTS AND DISCUSSION

The research objective utilizing NR and annual forage grazing as a component within a diverse multi-year, multi-crop rotation system was designed to increase calf value and system net return using a retained ownership vertically integrated business model through the growth spectrum from weaning to slaughter. Cropping systems designed with soil health improvement and resultant increased soil nutrient cycling as input reduction objectives, make crop diversity a priority, and

TABLE 1. Effect of frame score on extended grazing performance and cost[1]

	GRAZ[2] LF[3]	GRAZ[2] SF[3]	SEM[4]	*P*-value		
				Trt[5]	Yr[5]	Trt x Yr[5]
Number of steers	72.0	72.0				
Frame score	5.52[a]	3.77[b]	0.21	0.001	0.01	0.56
Winter corn backgrounding:						
Backgrounding days	163.00	163.00	0.59	0.18	<0.001	0.01
Start weight, kg	257.10[a]	205.30[b]	12.68	0.01	0.001	0.92
End weight, kg	353.90	305.80	17.73	0.38	0.02	0.86
Gain, kg	96.80	100.50	7.55	0.75	0.11	0.83
ADG, kg	0.59	0.62	0.04	0.80	0.05	0.95
Overall total performance:						
Grazed days	211.00	211.00				
Start weight, kg	353.90	305.80	17.73	0.38	0.019	0.86
End weight, kg	578.20[a]	509.80[b]	19.32	0.01	0.002	0.50
Gain, kg	224.30[a]	203.90[b]	4.97	0.04	0.07	0.27
ADG, kg	1.06[a]	0.97[b]	0.02	0.03	0.40	0.25
Grazing cost:						
Perennial pasture (108 Days), $[6]	115.30	100.24				
Field pea-barley (32 Days), $[6]	62.98	50.32				
Unharvested corn (71 Days), $[6]	110.81	88.53				
32% CP suppl. (0.37 kg/d), $	11.18	11.18				
Grazing cost/steer, $	300.27	250.27				
Grazing cost/kg gain, $	1.34	1.23				

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Three-year average.

[2]GRAZ steers grazed a forage sequence of native range, field pea-barley intercrop, and unharvested corn.

[3]SF; small frame, LF; large frame.

[4]SEM; pooled standard error of the mean.

[5]Trt - Treatment, Yr - Year, Trt x Yr - Treatment x Year.

[6]Grazing cost for SF steers adjusted by 20.1% based on the results of Senturklu et al. (2015).

animal waste from cattle grazing in the cropping system provides an added level of diversity.

Comparing LF and SF yearling steers during the 211 d grazing period (Table 1), SF steer growth rate was less (P = 0.03) during both grazing and feedlot finishing compared to the LF steers ($P < 0.001$). Frame score had a positive effect on grazing cost and grazing cost per unit of gain, which was less for the SF steers ($250.27 vs. $300.27/steer; $1.34 vs. $1.23/kg gain).

Delaying feedlot entry until after 211 d of grazing was associated with compensating steer ADG, reduced DOF (82 d), and finishing cost. In the feedlot following grazing (Table 2), LF steers had greater starting BW ($P < 0.001$), ending BW ($P = 0.003$), gain ($P < 0.001$), and ADG ($P < 0.001$). GRAZ steer compensatory gain, for the LF and SF steers, was 26.8 and 24.0% greater, respectively, compared to the LF and SF FLOT treatment steers. Feedlot gain to feed efficiency was numerically improved for GRAZ system steers compared to FLOT; however, the difference was not significant ($P = 0.72$). Comparing the average FLOT and GRAZ systems DM feed cost/kg of gain, finishing feed cost/kg gain for the GRAZ system averaged 34.0% less ($P = 0.001$).

Hot carcass weight (HCW, Table 3) was greater for LF steers in both systems Comparing systems LF steers, GRAZ LF steer HCW was greater than FLOT LF steers ($P = 0.01$). HCW for GRAZ SF steers was greater than FLOT SF steers (P = 0.01). Dressing percent was greater for SF steers in both FLOT and GRAZ treatments ($P < 0.001$) and SF steers had greater marbling score compared to the LF steers ($P = 0.02$). Ribeye area was greater for LF steers in both of the FLOT and GRAZ treatments ($P = 0.001$). Percent Choice or better quality grade ranged from 91.7 to 97.2%, but did not differ. Although the SF steers had greater marbling scores ($P = 0.02$) quality grade did not differ.

Meat tenderness measured using Warner-Bratzler shear force comparing FLOT and GRAZ treatments for LF and SF steers did not differ ($P = 0.48$). Meat cooking loss measured for FLOT and GRAZ treatments showed no treatment difference between FLOT and GRAZ ($P = 0.43$).

Economic analysis suggested that greater net return is realized, when ownership is retained through delayed feedlot finishing compared to selling the steers at the end of the 211d grazing period. Net return for

TABLE 2. Effect of steer frame score and extended grazing on feedlot finishing performance, efficiency, and economics[1]

	FLOT[2] LF[3]	FLOT[2] SF[3]	GRAZ[2] LF[3]	GRAZ[2] SF[3]	SEM[4]	*P*-value Trt[5]	Yr[5]	Trt x Yr[5]
Number of steers	72.0	72.0	72.0	72.0				
Frame score	5.63[a]	3.82[b]	5.53[a]	3.77[b]	0.26	<0.001	0.001	0.56
Growth performance:								
Grazing days	-	-	211.00	211.00				
Feedlot days Fed	218.00	218.00	82.00	82.00	3.51	<0.001	0.04	0.01
Start weight, kg	348.00	304.50	557.70	492.80	19.34	<0.001	<0.001	0.85
End weight, kg	687.60	595.20	730.20	635.40	23.56	0.003	<0.001	0.51
Gain, kg	339.6[a]	290.7[b]	173.10[c]	142.80[d]	7.63	<0.001	0.01	0.09
ADG, kg	1.56[c]	1.33[d]	2.11[a]	1.74[b]	0.054	<0.001	0.94	0.46
Feed intake and efficiency:								
DM feed/steer/day, kg	12.17	9.95	13.23	11.56	0.45	0.13	<0.01	<0.21
Gain:feed, kg	0.128	0.134	0.159	0.151	0.002	0.72	<0.056	<0.60
Finishing economics:								
DM feed, yardage, brand, and hospital cost/steer, $	674.98[a]	572.84[b]	247.56[c]	218.05[d]	11.71	<0.001	0.001	<0.001
DM feed, yardage, brand, and hospital cost/kg gain, $	1.99[a]	1.97[a]	1.43[b]	1.53[b]	0.022	<0.001	<0.001	0.02

[a–d] Within a row, means without a common superscript differ ($P < 0.05$).

[1]Three-year average.

[2]FLOT steers transferred directly to the University of Wyoming feedlot for growing and finishing; GRAZ steers grazed a sequence of native range, field pea-barley, and unharvested corn before transfer to the feedlot.

[3]SF; small frame, LF; large frame.

[4]SEM; pooled standard error of the mean.

[5]Trt - Treatment, Yr - Year, Trt x Yr - Treatment x Year.

TABLE 3. Effect of steer frame score and extended grazing on carcass trait measurements, value, and net return[1,2]

	FLOT[3] LF	FLOT[3] SF	GRAZ[3] LF	GRAZ[3] SF	SEM[4]	*P*-value Trt[5]	Yr[5]	Trt x Yr[5]
Carcass traits								
Hot carcass weight, kg	397.20[c]	349.30[d]	422.60[a]	373.30[b]	13.44	0.01	<0.001	0.01
Dressing percent, %	60.22[a]	61.09[b]	60.19[a]	60.84[b]	0.21	<0.001	<0.001	<0.001
Ribeye area, cm^2	84.70[a]	77.10[b]	89.90[c]	83.90[a]	4.05	0.001	<0.001	<0.001
Marbling score[6]	612.00[a]	640.70[b]	583.40[c]	631.40[ab]	10.21	0.02	0.01	0.21
Percent choice, %	93.10	94.24	91.67	97.22	2.73	0.11	0.04	0.19
Meat quality								
Warner-Bratzler shear Force, lb	2.43	2.41	2.64	2.64	0.061	0.48	<0.001	0.29
Cooking loss, %	17.85	17.61	17.50	15.40	1.17	0.43	<0.001	0.12
System economics								
Carcass value/steer, $	2073.33	1820.33	2223.67	1974.17	77.78	0.001	0.001	0.02
Total expenses[7]	1453.23	1255.27	1327.85	1152.36				
Net return	619.94	565.06	895.82	821.81				

[a–d] Within a row, means without a common superscript differ ($P < 0.05$).

[1]Three-Year average.

[2]FLOT steers transferred directly to the University of Wyoming feedlot for growing and finishing; GRAZ steers grazed a sequence of native range, field pea-barley, and unharvested corn before feedlot transfer. Slaughtered at Cargill Meat Solutions, Ft. Morgan, Colorado.

[3]SF; Small Frame, LF; Large Frame.

[4]SEM; Pooled Standard Error of the Mean,

[5]Trt - Treatment, Yr - Year, Trt x Yr - Treatment x Year.

[6]400 = small, 500 = modest, 600 = moderate

[7]Includes annual cow cost, steer backgrounding and grazing cost, feedlot cost, brand and health inspection, and transportation to feedlot and packing plant.

selling at the end of grazing was $514.02 and $642.90/steer for the GRAZ LF and SF steers, respectively. The SF steer margin at the end of grazing was $128.88 more than the LF steers. The SF steer profit advantage is attributed to the combined effect of 20% reduced annual cow cost, 20% greater carrying capacity, and reduced grazing and backgrounding costs. Overall, the three-year average systems net return/steer at the end of the feedlot phase was $619.94, $565.06, $895.82 and $821.81 for the FLOT LF and SF, and GRAZ LF and SF steers, respectively (Table 3). Net return for GRAZ LF and SF system steers was $275.88 and $256.75 greater than FLOT LF and SF steers. Regardless of frame score, grazing growing steers 211d before feedlot entry was more profitable than traditional feedlot growing and finishing. Profitability from the GRAZ system steers was due to a combination of reduced grazing and feedlot expenses, greater feedlot entry BW, compensatory growth and gain to feed efficiency, and increased HCW and value resulting in a system that was consistently more profitable.

IMPLICATIONS

The results of this 3-year study suggest that a long-term, yearling steer extended grazing system consisting of a combination of native range and annual forages increases feedlot entry BW and reduces the number of DOF without sacrificing carcass quality or meat tenderness, and is associated with consistently greater profitability.

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The effects of nitrogen addition on AC Saltlander forage production on saline soils[1]

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ABSTRACT: It has been suggested that growth limits and the negative effects caused by salinization can be mitigated by N fertilizer application. AC Saltlander (*Elymus hoffmannii*) (ACS) was developed at Agriculture and Agri-Food Canada - Swift Current Research and Development Centre (AAFC-SCRDC) to tolerate root-zone salinity with high quality and quantity. To evaluate the effects of N additions applied in the first year on forage production and quality of ACS on saline soil conditions, a consecutive 3-yr field experiment was carried out on a severe salinity field (EC_e > 16 dS/ m) at AAFC-SCRDC North Farm site during 2014 to 2016. A completely randomized block design was used with three N additions 0, 50, and 150 kg/ ha actual N. Results showed that: AC Saltlander yields at 50 and 150 kg/ ha were higher (P = 0.003 and P = 0.02) than 0 N addition and no differences between 50 and 150 kg/ ha N addition were observed in 2014 and 2015. However, no differences (P = 0.53) occurred among treatments in 2016. AC Saltlander yield was the highest in 2016 and the lowest in 2015 (P < 0.0001). Differences (P < 0.0001) were found for pH, NO_3^- and PO_4^- in every layer of soil (0-15, 15-30 and 30-60 cm) among years. For K^+ and ECe, differences (P < 0.001) were observed among years for the two bottom layers and the top layer, respectively. Interactions differences (P < 0.0001) were found for Zn, TN, TP and TK. Total N and TP increased with N additions and differences (P < 0.0001) among treatments in 2014 were observed. While TK in the top two N application treatments were similar, they both were higher (P < 0.0001) than 0 N addition. For Zn, only the 150 kg /ha N application was higher (P = 0.0003) than other treatments in 2014. No differences (P > 0.05) were found for Zn, TN, TP and TK among treatments in 2015. Interactions difference (P = 0.02) was observed for OMD. Nitrogen addition only increased (P = 0.0003) at 150 kg/ha N addition in 2014 and no other differences were observed for other years (P > 0.05). From the perspective of reducing production costs, a single large N application is not recommended to improve ACS forage yield and quality on severe saline soil conditions.

Key words: AC Saltlander green wheat grass, forage yield, nitrogen application, nutrient value, soil salinity

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INTRODUCTION

Nitrogen (N) is considered as one of the most important nutrients for plant growth, its deficiency reduces plant yield in globally terrestrial ecosystems (Lebauer and Treseder, 2008). Nitrogen fertilization plays an important role for increasing the productivity and nutrient value of crops and forages throughout the world (Duan et al., 2014). Nitrogen addition promotes plant photosynthesis by stimulating growth of leaves and branches and enhancing leaf area index, which results in more vegetative growth and overall biomass (Millard and MacKerron, 1986; Luo et al., 2015; Sher et al., 2016). Nonetheless, high chemical N application may give rise to soil salinization, poor soil structure and further adversely affect plant performance (Chen et al., 2010). Excessive use of fertilizer has contributed to a string of environmental contaminations (Duan et al., 2014).

Soil salinity is regarded as one of the major problems affecting extensive areas of land across the

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world (Wiebe et al., 2007; Huang et al., 2015). Plant establishment, growth and productivity are limited by excessive salinity (Rengasamy, 2010; Huang et al., 2015; Munns and Gilliham, 2015). It was estimated that 4 million hectare (10%) of cultivated lands within Canadian prairies are suffering salinization and caused around $250 million in economic losses each year and even increasing by at least $1 million annually (Dumanski et al., 1986; Wiebe et al., 2007). Salinity stress decreases plant growth mainly through osmotic stress, nutrient deficiencies or imbalances, as well as, specific ion toxicity (Huang et al., 2015). It has been suggested that growth limits and the negative effects caused by salinization can be mitigated if N fertilizer is applied properly, depending on plant species, salinity conditions, and environmental factors (Chen et al., 2010; Ding et al., 2010). Currently, numerous researchers have reported N addition can improve yields of crops and forages, as well as, their salinity tolerances (Liu et al., 2004; Yuan et al., 2010; Huang et al., 2015; Luo et al., 2015).

AC Saltlander (*Elymus hoffmannii*) (ACS) is the first cultivar of green wheatgrass developed at Agriculture and Agri-Food Canada - Swift Current Research and Development Centre (AAFC-SCRDC) to tolerate root-zone salinity and have high quality and quantity characteristics (Steppuhn and Asay, 2005; Steppuhn et al., 2006). It plays important roles in providing grazing and hay forage and the restoration of saline lands. Nitrogen addition may improve yield and nutrient value of ACS in severe salinity. Maximizing ACS forage yield and maintaining its quality while minimizing costs and environmental influence are urgently needed. The objective of this present study was to evaluate the effects of different N additions, applied once, on ACS forage production and quality over three production years on severe saline soil conditions.

MATERIALS AND METHODS

Experimental design and treatments

The experiment was conducted on a severe salinity field (EC_e > 16 dS/ m) at the AAFC-SCRDC North Farm site (50°17′50″ N, 107°45′16″ W, 817 m elevation) from 2014 to 2016. The soil was sandy-loam textured and classified as a Haverhill association. The site was staked for plot layout, and each plot was 12.19 m in length and 1.83 m wide. Prior to seeding 50 kg/ ha of 11-52-0 phosphate was broadcasted by a Valmar and worked in during cultivation. The cultivated site was harrowed-packed and AC Saltlander was seeded in 2009. Application of different N treatment with 46-0-0 urea started in 2014. A completely randomized block design was employed with three N application treatments. Each treatment had four replications (Fig. 1). The N addition levels were 0 (no fertilizer as control), 50 kg/ha of actual N (108.7 kg/ ha of 46-0-0) and 150 kg/ha of actual N (326.1 kg/ ha of 46-0-0). All fertilizers were broadcast homogeneously with a Press Drill before the rain was received on May 22th 2014.

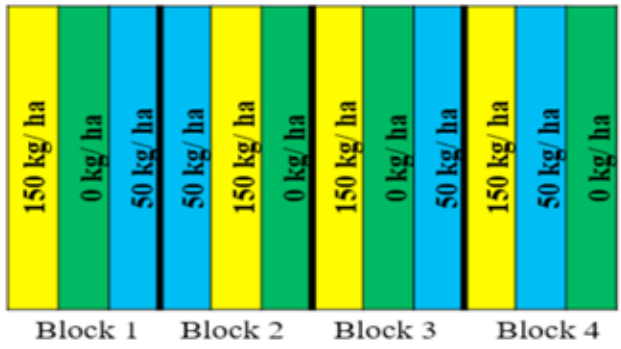

Figure 1. Schematic diagram for experimental plots and blocks.

Sample collection

Soil samples at 0-15, 15-30, 30-60 cm depth were collected prior to N addition on May 22th 2014 and also collected on April 16th 2015 and June 16th 2016. Three soil cores were taken randomly in each plot annually. Samples were air-dried, sieved through a 2 mm mesh screen and then the soil pH, electrical conductivities of the saturated paste extract (EC_e), K^+, NO_3^- and PO_4^- were measured. The K^+, NO_3^- and PO_4^- values were determined by an alkaline hydrolysis diffusion method, 0.5 mol/ L $NaHCO_3$ and 1mol/ L NH_4OAC extraction methods, respectively.

Four quadrats (1 m^2) were randomly sampled in each plot to determine forage yield in the middle of July from 2014 to 2016. AC Saltlander was clipped by scissors flush with the ground and bagged separately in each quadrat. All samples were oven-dried at 65 °C by 48 h to constant weight and then weighted. Then samples from 2014 to 2015 were triturated to determine total nitrogen (TN), total phosphorus (TP), total potassium (TK), S, Ca, Mg, Na and Zn, respectively. All samples from 2014 to 2016 were analyzed for organic matter (OM) and organic matter digestibility (OMD).

Statistical Analysis

Data were analyzed as a completely randomized block design using R software for windows (version 3.3.2). Normality and variance homogeneity of samples were determined by the Shapiro-Wilk test and the Barlett test respectively. Models for ACS included the effects of N addition, year, N addition and year interaction were conducted on biomass production,

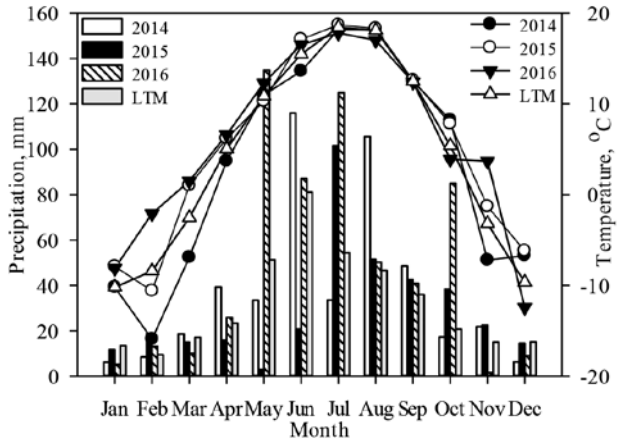

Figure 2. Monthly mean precipitations and air temperatures.

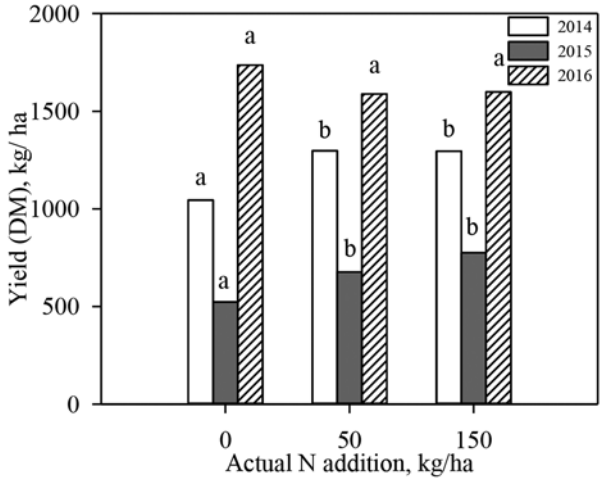

Figure 3. DM yields of AC Saltlander (ACS) from 2014 to 2016.

nutrient values and soil characteristics. Significance was declared at $P < 0.05$. The Tukey *t*-test was used to compare differences between multiple treatments.

RESULTS AND DISCUSSION

Weather

The annual long term mean (LTM) precipitation and temperature from 1983 to 2016 (34 years) were 383.9 mm and 4.3°C, respectively. From 2014 to 2016, the annual mean precipitations were 455.5, 356.2 and 588.3 mm, as well as, the annual mean temperatures were 3.2, 5.5, and 5.8°C, respectively. Figure 2 shows the monthly mean precipitations and air temperatures.

Yields of ACS

For ACS forage DM yield a significant N application by year interaction ($P = 0.03$) was observed. AC Saltlander yields at 50 and 150 kg/ ha were higher ($P = 0.003$ and $P = 0.02$) than the control (0 N addition) and no differences between 50 and 150 kg/ ha N addition were observed in 2014 and 2015. Nitrogen application had a positive impact on growth and yield (Luo et al., 2015; Sher et al., 2016). However, no differences ($P = 0.53$) occurred among treatments in 2016 and this may be due to N fertilizer being used up or lost. AC Saltlander yield was the highest in 2016 and the lowest in 2015 ($P < 0.0001$) (Fig. 3). The growth and production of ACS were more related to interannual variability and annual distribution of the precipitation. Low precipitation and higher growing temperature during the 2015 spring period reduced ACS production (Fig. 2). Precipitation in 2016 was 23% and 40% higher than 2014 and 2015, respectively and contribute to the higher yield.

Soil physical and chemical characteristics

All interactions associated with soil physical and chemical characteristics were not significant ($P > 0.05$), and only year main effect was significant. Differences ($P < 0.0001$) were found for pH, NO_3^- and PO_4^- in every layer of soil (0-15, 15-30 and 30-60 cm) among years. For K^+ and ECe, differences ($P < 0.001$) were observed among years for the two bottom layers and the top layer, respectively. It is unclear why the results showing differences among years at certain soil layers for certain soil physical and chemical characteristics occurred, but different environmental conditions were observed and may be a cause.

Forage nutrient content of ACS

No significant interactions ($P > 0.05$) were observed for S, Ca, Mg and Na, however differences ($P < 0.0001$) were observed among years. Interactions differences ($P < 0.0001$) were found for Zn, TN, TP and TK. Total N and TP increased with N additions and differences ($P < 0.0001$) among treatments in 2014 were observed (Fig. 4 and 5). While TK in the top two N application treatments were similar, they both were higher ($P < 0.0001$) than 0 N addition (Fig. 6). For Zn, only the 150 kg /ha N application was higher ($P = 0.0003$) than other treatments in 2014 (Fig. 7). No differences ($P > 0.05$) were found for Zn, TN, TP and TK among treatments in 2015. Nitrogen fertilization can increase plant N absorption and contribute to a significant increase in N concentration, and enhances the photosynthetic rate and the ability to adapt to drought stress in saline soils (Chen et al., 2010; Huang et al., 2015; Sher et al., 2016).

Interaction for OMD was significant ($P = 0.02$). Nitrogen addition only increased ($P = 0.0003$) OMD at 150 kg/ha N addition in 2014 and no other differences

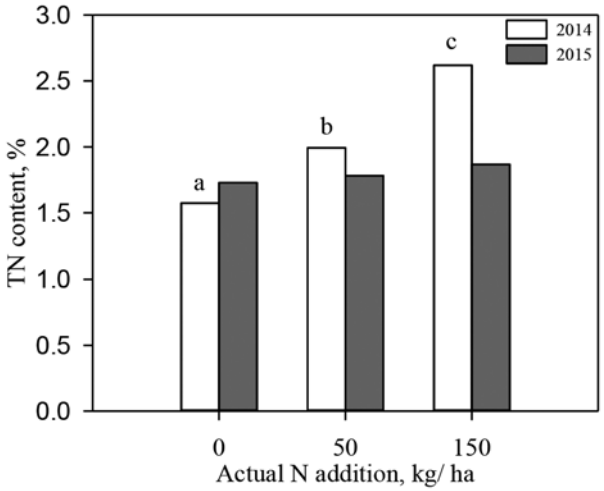

Figure 4. AC Saltlander (ACS) forage TN content.

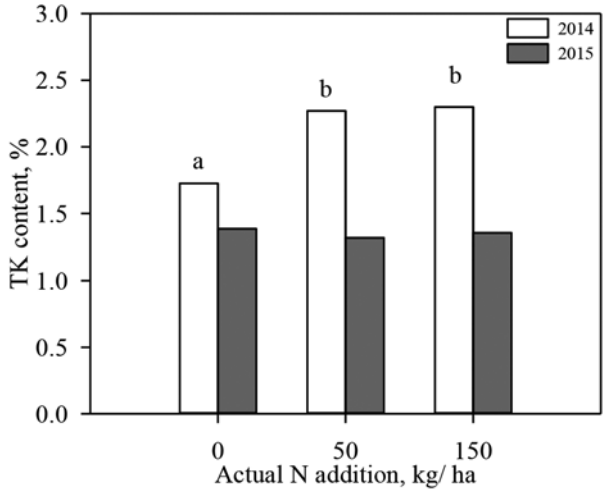

Figure 6. AC Saltlander (ACS) forage TK content.

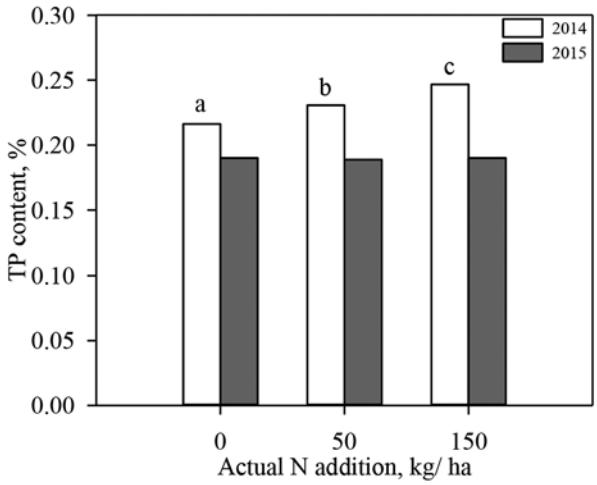

Figure 5. AC Saltlander (ACS) forage TP content.

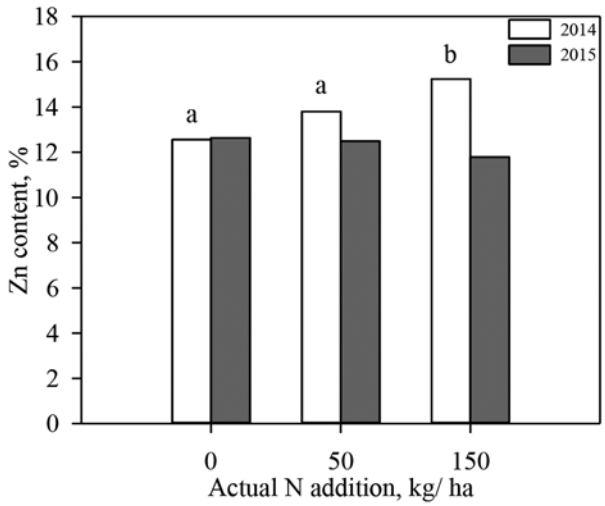

Figure 7. AC Saltlander (ACS) forage Zn content.

were observed for any other years ($P > 0.05$) (data not shown). It is not surprising the higher OMD might be observed for the higher N application during the 2014 application since certain forage nutrient contents like crude protein (CP) should be higher. Unfortunately all of the forage nutrient value (CP, cell wall constituents, etc.) have not been completely analyzed and further evaluation will be needed.

Implications

Our results showed no benefit to ACS forage production with a single large N application of 150 kg/ha versus 50 kg/ha. It also appears that the different N fertilizer applications were used up or lost after two production years, thus providing no benefits compared to the control (zero N application). Only TN, TP and TK forage nutrient content increased as N addition increased in the application year (2014). From the perspective of reducing production costs, a single large N application is not recommended to improve ACS forage yield and quality on severe saline soil conditions. Further research is needed to evaluate ACS forage performance over longer production years (> 5) and different environments and soil conditions need to be considered as well.

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Maternal inflammation at mid-gestation in pregnant rats impairs fetal muscle growth and development at term

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ABSTRACT: Intrauterine growth restriction (IUGR) is linked to lifelong deficits in muscle mass and metabolic function. Maternal stress increases fetal loss and causes developmental adaptations in fetal muscle, but little is known about the mechanistic role of inflammation in adaptive programming. Therefore, the objective of this study was to determine the effects of sustained maternal inflammation at mid-gestation on fetal mortality, muscle growth, and metabolic parameters at term. Timed-pregnant Sprague-Dawley rats were injected (i.p.) daily with saline (controls) or bacterial endotoxin lipopolysaccharide (LPS) on days 9-11 of gestation to induce maternal inflammation and then euthanized just before term (day 20). The average number of fetuses per litter did not differ between treatments, but fetal mass was lower ($P < 0.05$) in LPS rats. Fetal plasma TNFα tended to be greater ($P < 0.10$) and fetal hind limb muscle TNFR1 and IL6R mRNA tended to be decreased ($P < 0.10$) in LPS rats compared to controls. RNA markers for total macrophages (CD68) and M2 macrophages (CD163) also tended to be decreased ($P < 0.10$) in LPS fetal muscle compared to controls. CD68-positive nuclei were decreased ($P < 0.05$) in LPS fetal muscle but CD168-positive nuclei were not different between treatments. Moreover, myoD-positive nuclei (activated myoblasts) were decreased ($P < 0.05$) and myogenin-positive nuclei (differentiated myoblasts) tended to be increased ($P < 0.10$) in LPS fetal hind limb muscle compared to controls. Fewer total resident macrophages combined with greater plasma TNFα indicate that M1 fetal macrophages in LPS-treated rats were more productive despite reduced prevalence in skeletal muscle. Moreover, reduced muscle cytokine receptor expression is likely a compensatory response to sustained exposure to high concentrations of circulating cytokines that reduces muscle cytokine sensitivity. Lastly, the reduction in myoD-positive nuclei and increase in myogenin-positive nuclei indicates impaired myoblast function. Because myoblasts facilitate hypertrophic muscle growth, this is likely responsible for the decrease in fetal mass after maternal inflammation. Together, our findings demonstrate that maternal inflammation at mid-gestation causes fetal adaptations in muscle regulation that impair subsequent development and growth.

Key words: fetal hind limb, inflammatory cytokines
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INTRODUCTION

Intrauterine growth restriction (IUGR) is a leading cause of perinatal morbidity and mortality. Low birth weight resulting from preterm birth and/or IUGR is an underlying factor in 60-80% of perinatal death worldwide, and is particularly common in developing countries (UNICEF, 2008). Furthermore, studies have linked IUGR and the associated fetal malnutrition to increased incidence of metabolic syndrome in adult life (Barker et al., 1993; Godfrey and Barker, 2000). The "thrifty phenotype hypothesis" developed by David Barker (Hales et al., 1991) states that IUGR-associated fetal malnutrition forces the fetus to spare nutrients by altering tissue-specific metabolism in order to survive. In utero, adaptive changes disproportionately impact skeletal muscle development, growth, and metabolism (Yates et al., 2016). Skeletal muscle is respon-

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sible for the majority of insulin-stimulated glucose utilization, and adaptive restriction in muscle growth capacity helps to spare glucose in the IUGR fetus but result in lifelong deficits in muscle mass and metabolic homeostasis (Brown and Hay, 2016). Skeletal muscle growth requires proliferation, differentiation, and fusion of myoblast into new muscle fibers early in gestation and fusion with existing fibers in the third trimester of pregnancy (Zhu et al., 2004). This process can be impaired by inflammation from resident macrophages within skeletal muscle. Classically-activated M1 macrophages are pro-inflammatory but can polarize to an anti-inflammatory M2 phenotype that inhibits cytokine production and stimulates tissue repair by producing growth factors (Mantovani et al., 2004; Kharraz et al., 2013). The acute effects of inflammatory factors on myoblast function have been investigated in vitro (Frost et al., 1997; Guttridge et al., 2000), and we postulate that inflammatory stress may have similar effects on fetal myoblasts in utero. Impaired myoblast function and the resulting decrease in muscle growth capacity affect long-term metabolic health. Therefore, the objective of this study was to determine the effect of sustained maternal inflammation at mid-gestation on fetal mortality, muscle growth, and metabolic parameters at term.

MATERIALS AND METHODS

Animals and experimental design

Animal use and care was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Animal studies were performed at the University of Nebraska-Lincoln Animal Science Complex, which is accredited by the American Association for Accreditation of Laboratory Animal Care. From day 9 to 11 of gestation, time-mated Sprague-Dawley rats (Envigo, Indianapolis, IN) received daily i.p. injections of saline (n = 6) or 100μg/kg BW lipopolysaccharide (LPS, n = 7) from *E. coli* 055:B5 (Sigma-Aldrich). Rats were weighed daily, and maternal blood was collected and rectal temperature recorded throughout the treatment period. On day 20 of gestation, dams were euthanized by decapitation under heavy isoflurane anesthesia. Fetal mass and number were recorded and maternal and fetal blood samples were collected. Fetal hind limbs were collected from three randomly-selected fetuses per litter. For each fetus one hind limb was fixed in 4% PFA and the other was snap-frozen.

Blood analysis

Maternal and fetal blood glucose concentrations were determined at necropsy (Bayer Glucose Meter). Plasma was isolated by centrifugation (14,000 x g, 2 min) and TNFα concentrations were determined by Quantikine ELISA kit (R&D Systems) as previously described (Seo et al., 2017). Inter-assay CV was less than 10%.

Gene expression

Droplet digital PCR. RNA was extracted from fetal hind limb (30 mg) via RNeasy Fibrous Tissue Mini Kit (Qiagen), quantified by spectrophotometry (NanoDrop Technologies), and reverse transcribed via QuantiTect Reverse Transcription Kit (Qiagen). Primers for PCR were designed and droplet digital PCR (ddPCR) was performed with the QX200 ddPCR System (BioRad). Each reaction contained Evagreen Supermix, 10μM of each primer, and 1 μL of cDNA template. Droplets were generated in a QX200 Droplet Generator with Droplet Generator Oil, transferred to a PCR plate, sealed, and placed in a C1000 Touch Thermal Cycler. Samples were activated (95°C for 5 min), denatured for 40 cycles (95°C for 30 s), annealed and extended for 40 cycles (60°C for 1 min), and stabilized (4°C for 5 min and 90°C for 5 min). Finally, droplets were read on the QX200 Droplet Reader and results were analyzed with QuantaSoft Software to obtain copies/μL for genes of interest. Results for CD68, CD163, TNFR1, IL6R, insulin receptor β, and adrenergic receptors β1 and β2 were then normalized to the Ywhaz gene, which was shown to be stable across treatment groups.

Quantitative PCR. Expression of CD68 and CD163 was also measured via qPCR by the UNL Veterinary Diagnostic Center. RNA was extracted and cDNA was generated as described above. Template cDNA was standardized to 100 ng/reaction. Relative mRNA expression was determined using Power SYBR Green PCR Master Mix kits (Applied Biosystems) and ran on the Fast 7500 real-time PCR System. Samples were initially denatured (10 min at 95°C), followed by 40 cycles of 95°C for 15s, and an annealing and extension phase at 60°C for 1 min. mRNA expression was determined in triplicate from cDNA and normalized to the concentration of the housekeeping gene β-actin the using $2^{-\Delta\Delta Ct}$ method.

Immunohistochemistry

Fixed fetal hind limbs were embedded in OCT Compound (Thermo-Fisher) and 8μm cross sections were cut and mounted on glass microscope slides. Slides were dried at 37°C for 30 min and then thrice

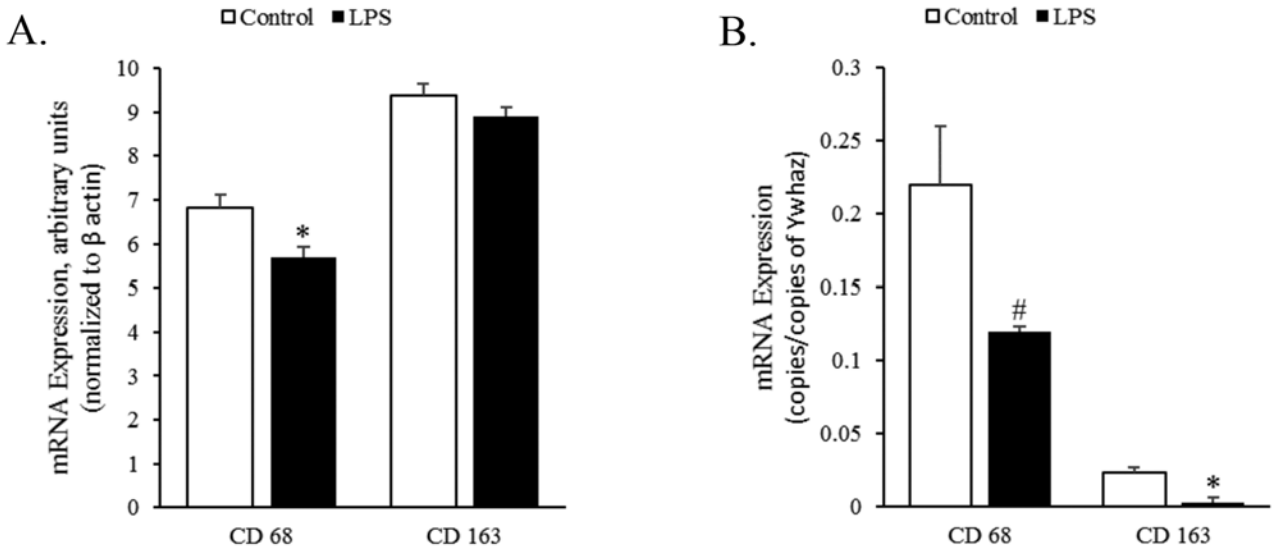

Figure 1. Gene expression analysis for total and M2 macrophage markers in fetal hind limb as measured by qPCR (A) and ddPCR (B) * means differed ($P < 0.05$) between control and LPS fetuses. # means tended to differ ($P < 0.10$) between control and LPS fetuses.

washed in PBS + 0.5% Triton-X-100. Antigen retrieval was performed by boiling slides in 10 mM citric acid for 20 minutes. Non-specific binding was blocked with 0.5% NEN blocking buffer (Perkin-Elmer) at room temperature for 1 hr. Slides were then incubated overnight at 4°C with primary antibodies diluted in PBS + 1% Bovine Serum Albumin. Negative controls were incubated without primary antibody. Sections were stained with rabbit antibody against myf5 (1:100; Santa Cruz), and mouse antibodies against myoD (1:200, Dako) and myogenin (1:250, Abcam) to identify nuclei expressing these myogenic factors. Macrophage profiles were determined by co-staining for total macrophages (CD68, 1:50; Abcam) and M2 macrophages (CD163, 1:100; Abcam). All nuclei were identified by counterstaining with DAPI (1:2000, Sigma-Aldrich). Immunocomplexes were detected with Alexa Fluor 594 (1:2000; Cell Signaling) or Alexa Fluor 488 (1:1000). Staining was visualized on an Olympus IX73 and digital micrographs were captured with a DP80 microscope camera (Olympus). Images were analyzed with CellsSens Dimension software to determine proportions of positive nuclei within fetal skeletal muscle sections. Animal identifications and treatments were encoded to eliminate bias.

Statistical analysis

All data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute) to determine treatment effects. Dam was the experimental unit. Skeletal muscle mRNA concentrations from ddPCR are expressed as copies per copy of Ywhaz. Macrophage mRNA concentrations analyzed by qPCR were normalized to β-actin and are expressed relative to the controls. All data are expressed as means ± standard error. Proportions of nuclei positive for myogenic factors and macrophage markers were determined from an average of 250 and 850 positive nuclei, respectively, counted across 18 fields of view.

RESULTS

Morphometrics and blood analysis

The number of fetuses per litter did not differ between control and LPS-treated rats, but fetal mass was reduced ($P < 0.10$) in LPS fetuses (71.4±2.8 and 60.8±2.8, respectively). Maternal blood glucose was reduced ($P < 0.05$) in LPS-treated rats 12 hours after the first daily injection but did not differ from controls otherwise (data not shown). Maternal plasma TNFα was greater ($P < 0.05$) in LPS-treated rats six hours after the first daily injection but did not differ from controls otherwise (data not shown). Fetal blood glucose at necropsy did not differ between treatments (data not shown), but LPS rats tended to have greater ($P < 0.10$) fetal plasma TNFα than controls (0.02±0.26 and 0.83±0.29 pg/ml, respectively).

Skeletal muscle gene expression

When measured by qPCR, CD68 mRNA expression was decreased ($P < 0.05$) in LPS fetal muscle but CD163 mRNA did not differ between treatments (Figure 1). When measured by ddPCR, CD68 mRNA concentrations tended to be decreased ($P < 0.10$) and CD163 concentrations were decreased ($P < 0.05$) in LPS fetal muscle compared to controls. TNFR1 and IL6R mRNA

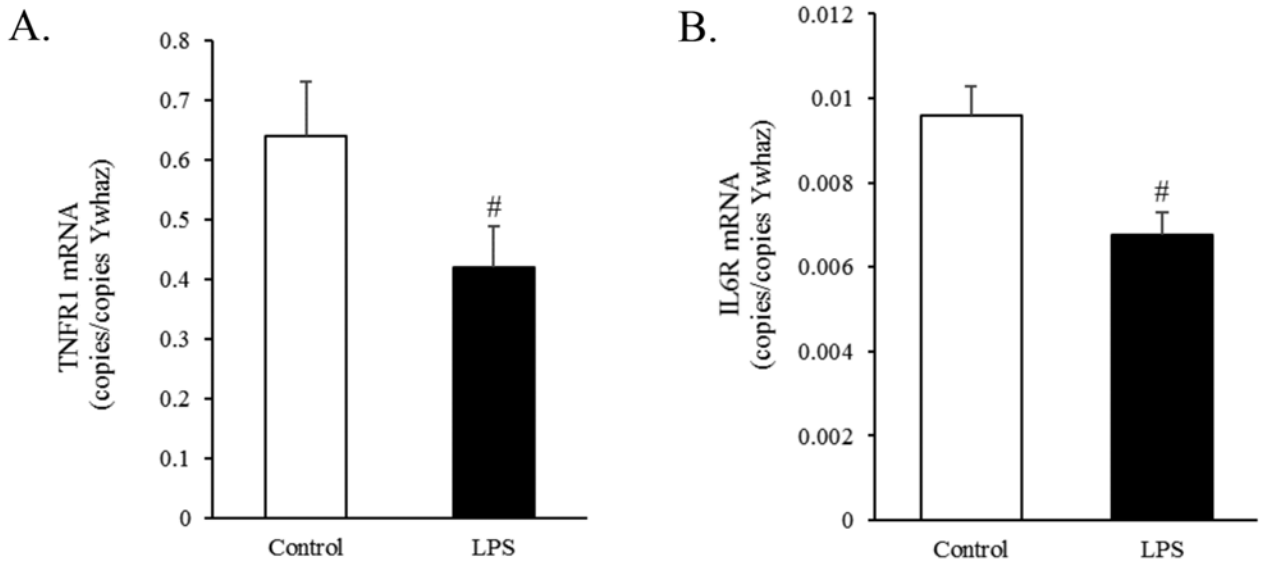

Figure 2. Gene expression analysis (ddPCR) of TNFR1 (A) and IL6R (B) in fetal hind limb after maternal inflammation. # means tended to differ ($P < 0.10$) between control and LPS fetuses.

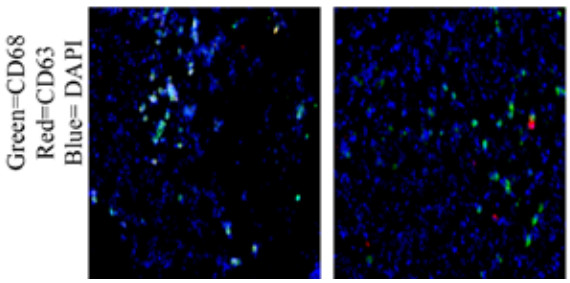

Figure 3. A. Immunostaining of markers for total (CD68) and M2 (CD163) macrophages in cross-sections of fetal hind limb muscle after maternal inflammation. A. Representative micrographs are depicted for control and LPS fetal hind limb cross sections (8μm). Sections were co-stained for total (green) and M2 (red) macrophages and counterstained with DAPI (blue). B. Analysis of positive total and M2 macrophages. # means differed ($P < 0.10$) between control and LPS fetuses.

tended to be reduced ($P < 0.10$) in LPS fetal muscle as well (Figure 2). No differences were observed for insulin receptor or β adrenergic receptor gene expression.

Skeletal muscle immunohistochemistry

CD68-positive nuclei/μm^2 tended to be decreased ($P < 0.10$) in LPS fetal muscle compared to controls but CD163-positive nuclei/μm^2 were not different (Figure 3). Likewise, the proportion of CD68-positive nuclei–to-CD163-postive nuclei did not differ between treatments (0.94±0.20 vs. 1.24±0.24, respectively). MyoD-positive nuclei/μm^2 were decreased ($P < 0.05$) and myogenin-positive nuclei tended to be greater ($P < 0.10$) in LPS fetal hind limb muscle (Figure 4), but myf5-postive nuclei/μm^2 did not differ between treatments (128.4±28.5 vs. 92.0±24.1, respectively).

DISCUSSION

In the present study, we show that reduced fetal growth is a consequence of maternal inflammation at mid-gestation. This decrease in fetal mass near term appears to be the result of restricted skeletal muscle growth capacity, as reduced myoD and increased myogenin in hind limb muscle was indicative of impaired myoblast function. Fetuses from LPS-treated dams had higher concentrations of circulating TNFα near term, which indicates that greater inflammation may be responsible for reductions in myoblast-induced fetal skeletal muscle growth. Additionally, increased circulating cytokines were accompanied by decreases in skeletal muscle TNFR and IL6R mRNA, which together with decreased myoD and increased myogenin indicate a compensatory decrease in cytokine sensitivity due to chronically high circulating inflammatory cytokines. These findings indicate that sustained maternal inflammation at mid-gestation impairs fetal skeletal muscle growth near term due to changes in myoblast responsiveness to critical cytokine regulation.

Fetal mass was decreased late in gestation following sustained maternal inflammation, likely due to decreases in skeletal muscle mass. We attribute decreased skeletal muscle growth to impaired myoblast function, as myoD was decreased and myogenin was increased in fetuses from LPS-treated dams. The absence of myoD results in impaired myoblast function, as myoblasts will continue to proliferate rather than exiting the cell cycle and fusing with fibers (Rudnicki et al., 1993). The combination of decreased myoD and increased myogenin indicates a greater percentage of differentiated myoblasts but a reduced percentage of active proliferating myoblast. This imbalance in MRF expression

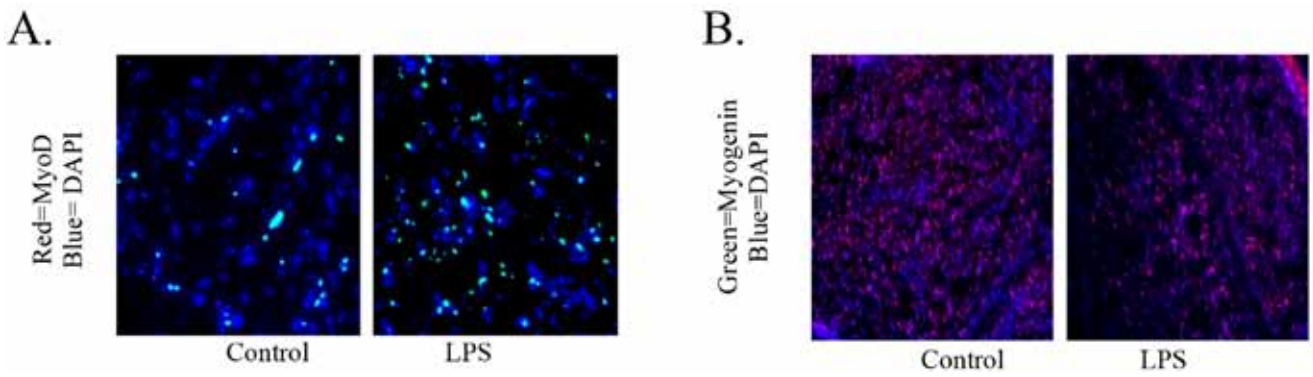

Figure 4. A. Immunostaining of myoD and myogenin in fetal hind limb muscles after maternal inflammation. Representative micrographs are depicted for control and LPS fetal hind limb cross sections (8 μm). Sections were stained for myoD (red) or myogenin (green) and counterstained with DAPI (blue). B. Analysis of myoD and myogenin positive nuclei. * means differed ($P < 0.05$) between control and LPS fetuses. # means differed ($P < 0.10$) between control and LPS fetuses.

represents an imbalance in the myogenic cell population and, ultimately, a deficient muscle growth capacity. Additionally, maternal inflammation resulted in higher concentrations of TNFα in fetal plasma, well after treatment ended. TNFα has been shown to impede differentiation and fusion of myoblasts at high concentrations (Miller et al., 1988). High fetal inflammatory cytokines reduced TNFR and IL6R in skeletal muscle, indicating a reduced sensitivity to these important regulators of skeletal muscle. Reduced sensitivity impedes the ability of cytokines to elicit a regulatory effect on myoblasts and causes an imbalance between proliferation and differentiation by allowing precocious differentiation.

Interestingly, increased circulating TNFα concentrations and decreased skeletal muscle cytokine sensitivity was not the result of greater numbers of resident macrophages, as mRNA and protein markers showed decreased total macrophage populations, but no difference in M2 macrophage population in fetal hind limb after maternal inflammation. However, these fetal macrophages are still producing greater amounts of inflammatory cytokines 10 days after maternal inflammation has subsided. Although it is not certain whether these inflammatory cytokines are from placental or fetal origin, the temporal spacing between induction of maternal inflammation and fetal plasma TNFα response suggest they are not of maternal origin. Moreover, since fetal TNFα is greater despite decreased macrophage number, it appears that fetal M1 macrophages have increased activity. As a compensatory action for chronic exposure to inflammatory cytokines in LPS fetuses, the major cytokine receptors were decreased in skeletal muscle. Inflammatory cytokines bind to these receptors and activate the NF-κB signaling pathway to upregulate gene expression of additional inflammatory cytokines, chemokines, and other inflammatory factors (Pahl, 1999). Activation of this pathway is responsible for the inhibitory effect of cytokines on myoblast differentiation and thus reduced sensitivity at the level of the receptor may explain the increase in myoblast differentiation and concurrent reduction in proliferating myoblast.

Fetal inflammation may be a direct response to maternal inflammation or may be indirectly caused by placental insufficiency. Maternal TNFα was acutely elevated in response to LPS treatment but did not differ from controls at day 20 of gestation when fetuses were collected, and in fact was not different on the second and third days of LPS administration. Fetal circulating TNFα was increased at term, suggesting fetal inflammation is most likely due to placental insufficiency. Additionally, previous studies found that inflammatory cytokines do not typically pass the placental barrier (Aaltonen et al., 2005), indicating that all cytokines within the fetus and amniotic fluid are of conceptus origin.

In conclusion, maternal inflammation at mid-gestation results in decreased fetal mass due to impaired myogenesis that is still apparent at term. We demonstrate that impaired myogenesis is due to myoblast dysfunction as evident by decreased myoD and increased myogenin. Moreover, myoblast dysfunction is the likely result of increased inflammatory cytokines and the resultant decreased sensitivity to these cytokines. The inflammatory response may be due to amplified activity of M1 macrophages, as macrophage number was actually decreased in fetal skeletal muscle after maternal inflammation. Together, our findings show that maternal inflammation induces fetal adaptive responses that interrupt myoblast regulation, causing myoblast dysfunction and impaired skeletal muscle development and growth.

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Restriction and realimentation in pregnant beef cows: Impacts on mammary gland hemodynamics

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ABSTRACT: The impacts of maternal diet on mammary gland development in beef cows are not well understood. Our hypothesis was that restriction, realimentation, or both, would impact mammary gland hemodynamics and endocrine status of the dam. On d 30 of pregnancy, multiparous crossbred beef cows were randomly assigned to dietary treatments: control (100% NRC; n = 6) and nutrient restriction (60% NRC; n = 11). On d 85, cows remained on control (n = 6) and restricted (n = 5) treatments, or were realimented to control (n = 6). On d 140, cows remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all cows were slaughtered. Doppler ultrasonography was used to obtain hemodynamic measurements of the external pudendal artery, which is considered to represent the majority of blood flow to the mammary glands. Blood samples were collected by jugular venipuncture to measure serum concentrations of progesterone, estradiol-17β, insulin-like growth factor, growth hormone, and prolactin. There was no effect ($P = 0.19$) of maternal diet on total mammary gland blood flow, although when analyzing the flow ipsilateral to the gravid horn, CCC cows had greater ($P \leq 0.02$) blood flow compared to RCC and RRC cows. No other hemodynamic measurements were affected. Endocrine status was not affected ($P > 0.50$) by maternal diet. There were minimal impacts on mammary gland hemodynamics and endocrine status due to restriction or realimentation. Further insights on how mammary gland development and milk production are driven by nutritional inputs need to be explored.

Key words: beef cow, mammary gland, nutritional status
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INTRODUCTION

Mammary gland development directly affects calf performance, not only due to colostrum production, which provides passive immunity, but also for milk production to nourish the calf until its greater dependence on solid feed. It is of interest to know how feeding management during critical times of mammary development, such as the peripubertal period, gestation, and lactation, can support mammary gland development and increase milk production in the dam (Purup et al., 2000).

We have previously reported (Camacho et al., 2013) that mammary gland weights and capillary vascularity are not influenced by nutrition during gestation. During late gestation, supplementing cows with dried distillers grains plus solubles (**DDGS**) along with ad libitum intake of low quality forage resulted in greater mammary gland blood flow during late gestation, and greater milk production during early lactation (Kennedy et al., 2016). It remains unknown how mammary gland blood flow is influenced by maternal diet during the majority of pregnancy. We hypothesize that cows experiencing restricted intake would have altered endocrine profiles that would modulate mammary gland blood flow and alter hemodynam-

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ics near term. The objectives of the study were to determine how maternal restriction and realimentation alters lactogenic hormones and mammary gland hemodynamics in multiparous beef cows from d 30 to 254 of gestation.

MATERIALS AND METHODS

Animals and Management

All procedures involving animals were approved by the North Dakota State University Animal Care and Use Committee (#A10001). Diets and animal procedures have been previously published (Camacho et al., 2014b). Briefly, multiparous crossbred beef cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) of similar genetic background were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and fixed-time AI. Inseminated cows were transported to the Animal Nutrition and Physiology Center (Fargo, ND) within 3 d post-insemination. On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). On d 30 of pregnancy, cows were randomly assigned to dietary treatments (n = 4 to 5 per pen with greater than 1 dietary treatment per pen): control (100% NRC; n = 6) and nutrient restriction (60% NRC; n = 11). On d 85, cows remained on control (n = 6) and restricted (n = 5) treatments, or were realimented to control (n = 6). On d 140, remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all cows were slaughtered (data not reported).

The control diet consisted of grass hay fed to meet 100% NE recommendations for maintenance and fetal growth (NRC, 2000) and to meet or exceed MP, mineral, and vitamin recommendations. Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system at 1000 h and had free access to water. The mineral and vitamin supplement (10% Ca, 5% Mg, 5% K, 2.7% Mn, 2.7% Zn, 1,565,610 IU/kg vitamin A, 158,371 IU/kg vitamin D 3 and 2,715 IU/kg vitamin E) was top-dressed 3 times per week at a rate of 0.18% of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC, 2000). Cows were weighed weekly at approximately 0800 h throughout the experiment and these data have been previously reported (Camacho et al., 2014b). Dietary intake was adjusted relative to BW weekly and to NE requirements for the specific period of gestation (average requirements for periods from d 30 to 85, d 86 to 140, 141 to 197, and d 198 to 254; Camacho et al., 2014b).

Starting on d 30 (prior to treatment implementation) and every 30 days until d 240, Doppler ultrasonography was employed to monitor mammary gland hemodynamics. Briefly, a 7.5-MHz finger probe was inserted rectally and used to identify the bifurcation of the internal and external iliac arteries. By following the latter, the external pudendal artery was identified. The external pudendal artery, which branches through the inguinal canal and continues branching to the udder, was measured and considered as representative of blood flow to the mammary glands (Budras et al., 2011; Mordhorst et al., 2016). The arteries were categorized as ipsilateral or contralateral to the pregnant uterine horn (as confirmed with uterine blood flow measurements; unpublished observations). Three separate cardiac cycle waveforms from 2 to 3 separate ultrasonography evaluations were selected for data collection and averaged (i.e., 6 to 9 measurements per artery per cow). Resistance index (**RI**), pulsatility index (**PI**), peak systolic velocity, end diastolic velocity, flow time, maternal heart rate (**HR**), mean velocity, blood flow, cross-sectional area (**CSA**) and cross-sectional diameter were recorded. The Doppler software was preprogrammed to calculate PI = (peak systolic velocity - end diastolic velocity)/mean velocity; RI = (peak systolic velocity - end diastolic velocity)/peak systolic velocity; and blood flow (mL/min) = mean velocity (cm/s) × CSA (cm^2) × 60 s/min. Finally, total mammary blood flow was calculated as the sum of ipsilateral and contralateral mammary blood flow measurements.

Blood sample collection and analyses

Upon completing the ultrasonography scans, blood samples were collected by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for 15 min before being placed on ice. Samples were centrifuged at 4°C for 15 min at 1,500 × g and serum was stored at −20°C in plastic vials until assayed. Serum concentrations of progesterone (P4), estradiol-17β (E2), insulin-like growth factor (IGF)-1, growth hormone (GH), and prolactin (PRL) were quantified by double-antibody RIA in Dr. Hallford's laboratory.

Statistical Analysis

Endocrine and mammary hemodynamics the repeated measures analysis of the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC) was used. The model included treatment, day, and treatment by day interaction. Breeding group was used as a block and appropriate covariance structures were selected. Data were analyzed using the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC). Class statement included cow and treatment. Model statement included treatment. When a

significant treatment effect was detected ($P \leq 0.05$), treatment differences were separated using the PDIFF option of the LSMEANS statement.

RESULTS

Mammary hemodynamics

There were no treatment × day interactions ($P \geq 0.21$) for any mammary gland hemodynamic measurements assessed. There was a main effect of diet ($P = 0.02$) for mammary gland blood flow ipsilateral, but not contralateral ($P = 0.21$) to the gravid horn where CCC had greater ($P \leq 0.02$) blood flow compared to RCC and RRC cows which did not differ ($P = 0.98$). When summed, there was no effect ($P = 0.19$) of maternal diet on total mammary gland blood flow. Maternal diet affected ($P \geq 0.19$) no other hemodynamic measurement.

All hemodynamic measurements were affected by day. Mammary gland blood flow increased ($P < 0.01$) as gestation advanced. Also, regardless of side, PI and RI measurements peaked ($P \leq 0.05$) on day 90 compared to other days assessed. Maternal heart rate increased ($P < 0.01$) from d 30 to 240 (58.7 vs 65.6 ± 1.7 beats per min).

Endocrine status

For the hormones analyzed in the current study, there were no treatment × day interactions ($P \geq 0.12$) or main effects of treatment ($P > 0.50$). As expected, there was a main effect of day ($P \leq 0.02$) for all hormones except GH which was constant throughout gestation ($P = 0.95$). As gestation advanced, E2 increased ($P < 0.01$) and IGF-1 decreased ($P = 0.02$). There was a peak of P4 on day 50 ($P < 0.01$) and PRL peaked at d 180 ($P < 0.01$).

DISCUSSION

While the current study was throughout gestation, our laboratory has published conflicting results. In a study where multiparous cows were supplemented DDGS to a diet where forage intake was limited, we found no change in mammary blood flow (Mordhorst et al., 2016). Most recently, we have demonstrated that when cows were allowed ad libitum forage intake with DDGS supplementation mammary gland blood flow was increased in supplemented dams (Kennedy et al., 2015). Therefore, mammary gland blood flow during pregnancy can be regulated by maternal nutrition.

When we restrict nutrient intake, we did not observe changes in uterine blood flow (Camacho et al., 2014a). Upon realimentation, however, those cows that were previously restricted have a greater uterine blood flow compared to those that never experienced a restriction (Camacho et al., 2014a). We did not see this pattern in the mammary gland data presented herein. While we approached a significant effect of treatment on total blood flow, on the ipsilateral side, we did observe that dams never experiencing a nutrient restriction had greater blood flow to the mammary gland compared to the other treatments. The time frame we saw the increased flow was at ~180 days of gestation. We have previously published that mammary glands from cows slaughtered at d 254 had similar mammary gland weights, but the CCC cows had a greater fat content compared to the RCC and RRC cows (22.1 vs 12.6 and 13.4 ± 2.37%; Camacho et al., 2013). It is unknown which factors may be specific to the mammary gland compared to uterine tissues that would drive blood flow. It is also unknown the milking potential for these dams. Our initial hypothesis was that hormonal factors known to grow and develop mammary tissue could account for alterations in mammary blood flow. The lack of endocrine changes resulting from different maternal diets supports the lack of dynamic hemodynamic differences observed.

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Biweekly Administration of Bovine Somatotropin during Early Pregnancy on Binucleate Cell and Conceptus Development in Beef Heifers

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ABSTRACT: The impact of biweekly administration of bovine somatotropin (bST; Sometirbove Zinc, Posilac) from d 0 to 77 of gestation on binucleate cells (BNC) and conceptus development was evaluated utilizing Angus heifers (n = 12). All heifers were subject to a 7d CO-Synch + controlled internal drug release (CIDR) ovulation control protocol with fixed-time artificial insemination (TAI). Timed AI and GnRH administration occurred on d0. Heifers were randomly assigned to one of two experimental treatments: subcutaneous bST injection every 14 days for 57 days (TRT; n = 7) or no bST injection (CON; n = 5). Bovine somatotropin (500 mg Sometirbove Zinc, Posilac) was administered via subcutaneous injection in the neck on d 0, 15, 29, 43, and 57 of gestation. Transrectal ultrasonography was utilized to confirm pregnancy status on d 29 and d 63 after TAI. Heifers were transported to a commercial abattoir and were harvested on d 77 of gestation. Conceptus samples were collected immediately following harvest. Fetal fluid weight, fetal fluid volume, fetal weight, umbilical cord diameter, total placentome weight, total placentome number, and fetal membrane weight were recorded. One placentome per heifer was collected and fixed in formalin for histological analysis. Experimental treatment had no impact on BNC number ($P = 0.13$), BNC size ($P = 0.19$), percent BNC per cotyledon area ($P = 0.25$). Conceptus weights did not differ ($P \geq 0.24$). Total placentome number tended to be reduced ($P = 0.08$) in TRT compared to CON, whereas umbilical cord diameter and fetal fluid volume tended to be ($P = 0.09$) or was ($P = 0.03$) greater in TRT compared to CON (Sanford et al., 2017). Results indicate bST administration during early pregnancy in beef heifers will increase fetal fluid volume, yet has little impact on BNC parameters or conceptus measurements by the end of the first trimester.

Key words: binucleate cell, bovine somatotropin, placentome
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INTRODUCTION

During placental development, chorionic villi interdigitate with the maternal caruncles to form placentomes (Lawn et al., 1969). During implantation, chorionic and uterine luminal epithelial cells form a syncytium, to form BNCs (Bazer et al., 2010). Bovine BNCs have granular tissue that contain lactogens which are released into maternal tissues throughout gestation (Alvarez-Oxiley et al., 2008). While bovine BNCs release placental lactogen (PL), also known as choriosommatomammotrophin (CSH), directly to maternal tissues, bovine fetuses have a higher concentration of PL compared to ovine (Ogren and Talamantes, 1988). Milosavljevic et al. (1989) were the first to discover that bovine PL mRNA is found only in the placentome of bovine. Wooding (1992) later concluded that ruminant BNCs are the sole source of PL within the placentae. In cattle, fetal PL concentration and fetal weight are positively correlated as well as fetal weight and placental weight (Schoknecht et al., 1991). Hossner et al. (1997) reported a positive correlation between maternal PL and fetal weight in calves.

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Directly increasing PL through an increase in BNCs is not the only way to increase fetal weight and growth in calves. Infusion of growth hormone (GH) in overnourished, early pregnancy ewes at breeding resulted in greater uteroplacental weight at d 35 to 80 (Wallace et al., 2004). Costine et al. (2005) concluded that GH administration at time of breeding in ewes had a greater cotyledonary weight by mid gestation. While placental weights were similar near term, lamb birth weights were increased by 10% when ewes were administered GH at time of breeding (Costine et al., 2005; Koch et al., 2010).

The objective of this study was to quantify the impact of bST administration on BNC number, size, and percentage of BNCs within the placentome, as well as conceptus measurements. Our hypothesis was that bST treated heifers would have an increase in BNC numbers formed, have greater placental growth, and therefore have heavier calves by the end of the first trimester of gestation in beef cattle.

MATERIALS AND METHODS

Experimental Design

All procedures were approved by the University of Florida Animal Care and Use Committee (IACUC Number: 201609600). Angus heifers ranging in the age of 15 to 18 months at time of breeding with an initial body weight of 430 ± 21.30 kg were housed on pasture 19A at the University of Florida North Florida Research and Education Center located in Marianna, FL. Pasture consisted of bahiagrass (*Paslpalum notatum*) and all heifers were fed *ad libitum* supplementation consisting of 10% fiber pellets; 41.2% corn gluten; 40.8% soy hull pellets; 5% vitamin and mineral supplement; and 3% whole soybeans. Trifton85 bermuda (*Cynodon dactylon*) grass hay, water, and mineral supplement were provided *ad libitum*. All heifers were subject to a 7d CO-Synch + controlled internal drug release (CIDR; 1.38 g of progesterone) ovulation control protocol with fixed-time artificial insemination (TAI). Briefly, gonadotropin releasing hormone (GnRH) was administered to heifers on d -9, relative to TAI, when the CIDR was placed intravaginally to release progesterone. Prostaglandin F2α (PG) administration and CIDR removal occurred on d -3 relative to TAI (d 0). Timed AI and GnRH administration occurred on d 0 and heifers were randomly assigned to one of two experimental treatments: subcutaneous bST injection (500 mg Sometirbove Zinc, Posilac; Elanco Animal Health; Indianapolis, IN, USA) every 14 days for 57 days (TRT; n = 7) or no bST injection (CON; n = 5), which served as the control. Bovine somatotropin (Sometribove Zinc, Posilac) was administered via subcutaneous injection in the neck on d 0, 15, 29, 43, and 57 of gestation. Transrectal ultrasonography was utilized to confirm pregnancy status of heifers on d 29 and d 63 after TAI. Heifers were transported to a commercial abattoir and were harvested on d 77 of gestation.

Sample Collection

Plant personal collected complete gravid reproductive tracts immediately following harvest. Fetal fluid weight, fetal fluid volume, fetal weight, umbilical cord diameter, total placentome weight, and total placentome number were obtained immediately postmortem. Fetal fluid weight, fetal membrane weight, and fetal weight were measured utilizing a scale while umbilical cord diameter was obtained utilizing a digital caliper. One placentome near the umbilicus was selected per heifer; trimmed to a 1 cm cube; and fixed in formalin for histological analysis. Placentomes were stored in a 70% ethanol (ETOH) solution prior to being embedded in paraffin.

Immunofluorescence Staining

Placentome samples were trimmed to a width of 0.50 cm and embedded in paraffin to be utilized for placentome sectioning (3 µm thickness). Placentome sections were deparaffinized, rehydrated in descending concentrations of ETOH (100%, 95%, 70%, and 50% ETOH), and rinsed in distilled water. Heat induced antigen retrieval was performed utilizing 10mM sodium citrate buffer (pH = 6.0) and were exposed to 121ºC for 20 min under pressure to expose target proteins (2100 Antigen Retriever; Aptum Biologies; Southampton, UK). Tissues were blocked in 10% normal goat serum (NGS; Vector Labs; Burlingame, CA, USA) for 60 min at 25ºC. Placentome sections were incubated with 20µg/ml biotinylated lectin Dolichos Biflorus Agglutinin (DBA; Vector Labs; Burlingame, CA, USA) at similar conditions. Texas red-avidin (20µg/ml; Vector Labs; Burligame, CA, USA) was added to tissues and tissues at 25^0C for 60 mins in a dark room under similar conditions. This was completed to allow for fluorescent staining of BNCs. Next, 20µg/ml Fluorescein labeled Griffonia (Bandeiraea) Simplicifolia lectin I (BS1-FICT; Vector Labs; Burlingame, CA, USA) was added to tissues and again, tissues were held at similar condition. This was completed to allow for fluorescent staining of cotyledon area. All reagents were diluted in a mixture of 1x tris buffered saline (TBS) and 1% NGS and prepared at least 15 min prior to addition to the slides.

Imaging and Image Analysis

Five representative images of each slide were acquired with ZEN 2 pro software using a Zeiss Inverted AxioObserved.Z1 Microscope equipped with Plan-Apochromat 20x objective and Axiocam 506 monochrome camera (Carl Zeiss; Thornwood, NY, USA). Image analyses were performed using Image-Pro Premier 9.1 software (Media Cybernetics; Rockville, MD, USA) to obtain BNC number and cotyledon area from each slide. Analysis was conducted on each images per slide and averaged. The average cotyledon and BNCs number per heifer were used to calculate percentage BNCs per cotyledon area.

Statistical Analysis

Data was analyzed using the MIXED procedure in SAS (v. 9.4; SAS Inst. Inc.; Cary, NC, USA) using LSMEANS with PDIFF function. Two tissues samples were removed from the study as one tissue was biologically inconsistent and the other tissues collected was not placental tissue. Binucleate cells and conceptus parameters were analyzed using CORR procedure in SAS (v. 9.4; SAS Inst. Inc.; Cary, NC, USA). Experimental unit was individual heifer. Significance was defined at $P \leq 0.05$ and tendency was defined at $0.10 \leq P \geq 0.05$.

RESULTS

Experimental treatment had no impact on BNC number ($P = 0.13$), BNC size ($P = 0.19$), percentage BNC per cotyledon area ($P = 0.25$), total placentome weight ($P = 0.24$), fetal membrane weight ($P = 0.90$), or fetal weight ($P = 0.85$). Total placentome number tended to greater ($P = 0.08$) in CON compared to TRT (Table 1). Umbilical cord diameter tended to be greater ($P = 0.09$) in TRT compared to CON (Table 1). Fetal fluid volume was greater ($P = 0.03$) in TRT compared to CON (Table 1).

TABLE 1. Effects of experimental treatment on binucleate cell and conceptus development in beef heifers

Item	Treatment[1]		SEM	*P*-value
	CON	TRT		
BNC number	98.20	112.57	5.90	0.13
BNC size, µm	441.14	524.46	40.95	0.19
% BNC per cotyledon area	9.43	11.45	1.14	0.25
Fetal membrane weight, kg	0.27	0.25	0.13	0.90
Total placentome number[2]	73.60	54.71	6.92	0.08
Total placentome weight,[2] g	73.62	61.35	6.85	0.24
Fetal fluid volume,[2] mL	429.60	521.57	25.04	0.03
Fetal weight,[2] g	60.09	59.40	2.50	0.85
Umbilical cord diameter,[2] mm	6.66	8.90	7.78	0.09

[1]Treatments: subcutaneous bST injection every 14 days for 57 days (TRT; n = 7); no bST injection (CON; n = 5).

[2]Sanford et al., 2017.

DISCUSSION

Results indicate that bST treatment tended to decrease total placentome number; however, BNC number is approaching (P = 0.13) significance with an increase of BNCs due to bST treatment. Also, total placentome weight is not different between the groups. It should also be noted that Costine et al. (2005) found no difference in early (d 25) pregnancy chorioallantoic weight due to GH administration at time of breeding in well fed sheep, but they did observe cotyledonary differences by mid gestation (d 80). Perhaps we would have observed differences in placentome numbers by mid pregnancy. There is the potential that bST treatment during early pregnancy could enhance BNC numbers and perhaps placentome growth to aid in nutrient delivery to the calf. The observed tendency for an increase in umbilical cord diameter due to bST treatment suggests that nutrient delivery may increase as gestation continues.

An increased number of BNCs are expected to increase PL secretion and therefore fetal weight (Schoknecht et al., 1991; Hossner et al., 1997). The present study indicated that there is no change in fetal weight during early pregnancy, which contradicts previous findings (Wallace et al., 2004). These differences could be due to species differences or the time of pregnancy these observations were recorded.

An increase in fetal fluid volume with no difference in fetal membrane weight and fetal weight due to GH has also been observed in over nourished sheep (Wallace et al., 2004). While our heifers were not intentionally over nourished, we are currently unaware of the endocrine status of the dam, which has been shown to be affected by maternal nutrient status (Lemley et al., 2014). Perhaps bST administration alters placental steroid hormone production and hepatic clearance. Studies are currently underway to test this.

IMPLICATIONS

Results indicate bST administration during early pregnancy in beef heifers will increase fetal fluid volume, yet has little impact on BNC parameters or conceptus measurements by the end of the first trimester. Binucleate and conceptus outcomes were inconsistent with our hypothesis and were not as responsive to bST administration as originally thought. However, there is indication for enhanced BNC number and conceptus growth as gestation continues. Additional research is needed to further define the impact of bST administration in early pregnancy

along with mid to late pregnancy on binucleate and conceptus development.

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Relationships of circulating energy and protein metabolites from 0 to 72 hours of age in suckling neonatal beef calves from fall- and spring-calving herds

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ABSTRACT: The objectives of this study were to 1) determine changes over time in neonatal calf circulating glucose, NEFA, blood urea nitrogen (BUN), total protein, and globulin at 0, 6, 12, 24, 48, and 72 h of age; and 2) evaluate the relationships of sampling times within each metabolite. Three experiments were conducted at the University of Missouri Beef Research and Teaching Farm using calves described. Exp. 1 was conducted in fall-calving cows, with Exp. 2 and 3 conducted in spring-calving cows. Calf blood samples were obtained from a subset of calves in each experiment based on time of birth and cow temperament. Jugular blood samples were obtained from calves at 0, 6, 12, 24, 48, and 72 h postnatally. Blood samples collected at 0 h were obtained prior to suckling but after standing. Data were first analyzed with effects of experiment, sampling hour, and their interaction in the model, using sampling hour as a repeated effect. An experiment effect that appeared to be driven by calving season was detected for some metabolites; therefore, correlations between each sampling hour pair were determined within each metabolite separately for fall and spring-born calves using. Serum glucose and NEFA were affected by the interaction of experiment and sampling hour ($P \leq 0.005$). Spring-born calves had greater ($P < 0.05$) serum glucose at 0, 12, 24, and 48 h than fall-born calves. Fall-born calves had less ($P < 0.05$) NEFA at 0 h, but greater ($P < 0.05$) NEFA at 12 h. Serum BUN was affected by the main effects of experiment ($P < 0.001$) and sampling hour ($P < 0.001$). Serum total protein and globulin were affected by sampling hour ($P < 0.001$). Metabolites at 0 h were correlated with few other sampling times, which may be due the changes in metabolites over time and the difference in pre- and postnatal delivery of nutrients. There was a greater number of positive relationships in metabolites from consecutive sampling times and may be due to the similar intake of colostrum or milk after the initial intake of the postnatal nutrients, especially after 24 h of age. In conclusion, neonatal calf energy and protein metabolites change during the first 72 h of age, and may be affected by calving season. Additionally, these data demonstrate that a consistent sampling time is necessary due to the changes in metabolites over time in neonatal beef calves.

Key words: beef cattle, metabolism, neonate
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INTRODUCTION

Circulating metabolites and immunoglobulin concentrations are often determined in neonatal calves as an indication of pre- or postnatal nutrient availability. It can be extremely time- and labor-intensive to collect neonatal calf blood samples at specific hours of age, or is often impractical to collect time of birth in more extensive studies. For these reasons, some researchers elect to collect blood samples within a range of ages (e.g. within 24 h postnatal) or at a specific postnatal age (e.g. 48 h). There are currently minimal data from healthy, suckling beef calves to provide reference ranges or changes over times for investigators to use when choosing sampling times.

Recent work in our laboratory indicates that neonatal beef calf metabolites can change dramatically in the first 72 h of life (Larson, 2016), yet the relationship of metabolites across these sampling times is unknown. The objectives of this study were to 1) determine changes over time in neonatal calf circulating glucose, NEFA, blood urea nitro-

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TABLE 1. Descriptive statistics of cows and calves for each experiment (± SD)

Item	Exp. 1 (Fall 2015)	Exp. 2 (Spring 2016)	Exp. 3. (Spring 2017)
Total n	24 (16 heifers, 8 bulls)	31 (17 heifers, 14 bulls)	28 (11 heifers, 17 bulls)
Cow age, yr	4.5 ± 2.6	2.3 ± 2.4	3.1 ± 2.9
Cow parity	6 primiparous, 18 multiparous	All multiparous	13 primiparous, 15 multiparous
Cow BCS	5.2 ± 0.1	5.1 ± 0.6	5.2 ± 0.5
Gestation length, d	280 ± 3	277 ± 4	276 ± 3

gen (BUN), total protein, and globulin at 0, 6, 12, 24, 48, and 72 h of age; and 2) evaluate the relationships of sampling times within each metabolite.

MATERIALS AND METHODS

All procedures were approved by the University of Missouri Animal Care and Use Committee. Three experiments were conducted at the University of Missouri Beef Research and Teaching Farm using calves described in Table 1. Exp. 1 was conducted in fall-calving cows, with Exp. 2 and 3 conducted in spring-calving cows. In each study, pregnant heifers and/or cows were transported to 18 x 61 m uncovered drylots prepartum for intensive monitoring during calving. Cows were allowed to calve outside, except in cases of extreme cold or wind. Calves that were born during inclement weather were moved to adjacent covered pens for approximately 6 h, and then moved back to the uncovered drylot. All calves used in the present analysis were from AI mating on a single date in each experiment.

No prepartum treatment diets were fed in Exp. 1 and 3, but cows in Exp. 2 were part of a study to determine effects of forage system during late gestation (stockpiled tall fescue vs. poor quality tall fescue hay). Because few effects of gestational forage system were observed in calf serum metabolites in Exp. 2, all animals were included in this analysis.

Calf blood samples were obtained from a subset of calves in each experiment based on time of birth and cow temperament. Jugular blood samples were obtained from calves at 0, 6, 12, 24, 48, and 72 h postnatally. Blood samples collected at 0 h were obtained prior to suckling but after standing. At each sampling time, blood samples were collected to obtain both serum and plasma using Vacutainer® serum collection tubes containing no additives [10 mL draw; Becton Dickinson, Franklin Lakes, NJ] and 1 Vacutainer® plasma collection tube containing 15 mg of sodium fluoride and 12 mg of potassium oxalate for glucose determination [6 mL draw; Becton Dickinson, Franklin Lakes, NJ]. Plasma tubes were inverted as directed and placed on ice immediately following collection, and serum tubes were inverted but allowed to clot prior to placing on ice. Samples were centrifuged at 1500 x g at 4°C for 30 min within 8 h of collection. Serum or plasma was then pipetted into 2 mL microcentrifuge tubes and stored at 4°C or -20°C until analysis.

Calf serum was refrigerated and transported to the University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL) for a complete chemistry profile analysis of glucose, blood urea nitrogen (BUN), total protein, globulin, and other blood chemistry measures. Samples were analyzed using a Beckman Coulter AU 400e Chemistry System (Beckman Coulter Inc., Brea, CA) on the day of collection, or stored at 4°C when collected on evenings or weekends until analysis within 72 h of collection.

Serum samples in Exp. 1 were analyzed for BUN using a commercially available urea nitrogen kit (Urea Nitrogen Procedure Number 0580; Stanbio Laboratory, Boerne, TX) based on the diacetylmonoxime method. Glucose concentration was determined in plasma samples (collected in treated tubes described above) from Exp. 1 using the InfinityTM glucose hexokinase commercially available kit (Cat. # TR15421, Fisher Diagnostics, Middletown, VA) based on the glucose-6-phospate dehydrogenase method. Samples were read in duplicate in 96-well polystyrene plates (Corning Inc., Corning, NY) on a microplate reader (Biotek SynergyTM HT, Biotek® Instruments Inc., Winooski, VT) following kit manufacturer instructions. The BUN intraassay and interassay CV were 2.84% and 3.38%, respectively. The glucose intraassay and interassay CV were 4.15% and 4.39%, respectively. Serum BUN values determined using kit and performed by and serum or plasma glucose were highly correlated (r^2 = 0.82). Plamsa glucose (kit) and serum glucose (VMDL) were also highly correlated (r^2 = 0.94). Therefore, VDML analyses were used exclusively in Exp. 2 and 3.

Serum samples from Exp. 1 and 2 were analyzed for NEFA concentrations using a modified procedure of the NEFA C kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan), using the acyl-CoA synthetase-acyl-CoA oxidase method. Samples were read in duplicate in 96-well plates as described above at 550 nm. The intraassay and interassay CV were 4.09% and 8.16%, respectively for Exp. 1, and 4.20% and 4.74%, respectively for Exp. 2.

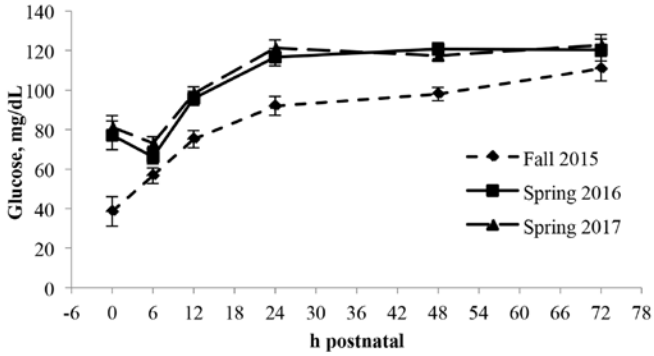

Figure 1. Effects of experiment ($P < 0.001$), sampling hour ($P < 0.001$), and their interaction ($P = 0.005$) on neonatal calf serum glucose.

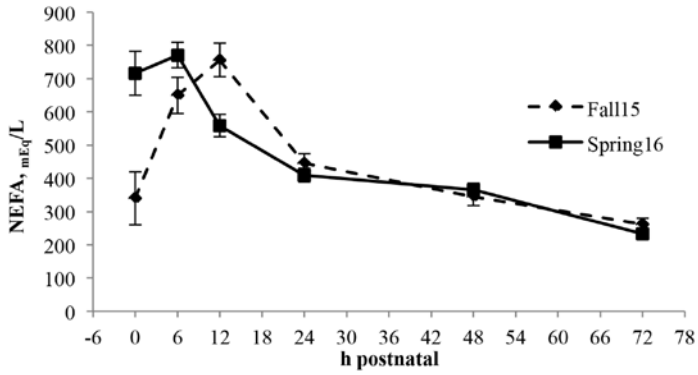

Figure 2. Effects of experiment ($P = 0.015$), sampling hour ($P < 0.001$), and their interaction ($P < 0.001$) on neonatal calf serum glucose.

TABLE 2. Effects of sampling hour on serum BUN, total protein, and globulin in neonatal calves

	Sampling hour, postnatal age							
Item	0 h	6 h	12 h	24 h	48 h	72 h	SEM	*P*-value
BUN	8.49^{e}	9.98^{cd}	10.75^{b}	11.29^{a}	10.35^{bc}	9.64^{d}	0.55	<0.001
Total protein	4.09^{d}	5.20^{c}	6.37^{b}	6.72^{a}	6.49^{b}	6.39^{b}	0.11	<0.001
Globulin	1.48^{e}	2.83^{d}	4.23^{bc}	4.64^{a}	4.31^{b}	4.14^{c}	0.12	<0.001

Data were first analyzed using the MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC) to determine effects of experiment, sampling hour, and their interaction on circulating metabolites. Data were analyzed using sampling hour as a repeated effect. Sampling hour was highly significant for all measures ($P < 0.001$). An experiment effect that appeared to be driven by calving season was detected for some metabolites; therefore, correlations between each sampling hour pair were determined within each metabolite separately for fall and spring-born calves using PROC CORR.

RESULTS AND DISCUSSION

Effects of Sampling Hour and Experiment

Serum glucose (Figure 1) and NEFA (Figure 2) were affected by the interaction of experiment and sampling hour ($P \leq 0.005$), whereas BUN was affected by the main effect of experiment ($P < 0.001$; Table 2). Additionally, total protein and globulin were affected by sampling hour ($P < 0.001$; Table 2). Overall, sampling hour had a greater effect than experiment, as we have previously reported (Larson, 2016). Despite this, energy metabolites responded differently to sampling hour within season. Spring-born calves had greater ($P < 0.05$) serum glucose at 0, 12, 24, and 48 h than fall-born calves. Additionally, fall-born calves had less ($P < 0.05$) NEFA at 0 h, but greater ($P < 0.05$) NEFA at 12 h. This suggests that energy metabolism differs in fall- and spring-born calves, possibly due to ambient temperature at birth.

Relationship Among Sampling Times

Correlations of postnatal calf sampling time for serum BUN, plasma glucose, serum total protein, and serum globulin are presented in Tables 3 and 4 for fall-born and spring-born calves, respectively.

TABLE 3. Partial correlation of coefficients of postnatal calf sampling time for serum blood urea nitrogen, plasma glucose, serum total protein, and serum globulin concentrations between sampling hours in fall-born calves (Exp. 1)

	Sampling hour, postnatal age					
Items	0 h	6 h	12 h	24 h	48 h	72 h
Plasma glucose, mg/dL						
0 h	--	0.69**	-0.07	0.02	0.39*	0.11
6 h	0.69**	--	0.45*	0.43*	0.38*	-0.29
12 h	-0.07	0.45*	--	0.59**	0.04	-0.47*
24 h	0.02	0.43*	0.59**	--	0.34	0.15
48 h	0.39*	0.38*	0.04	0.34	--	0.24
72 h	0.11	-0.29	-0.47*	0.15	0.24	--
Serum NEFA, mEq/L						
0 h	--	-0.12	0.00	-0.11	-0.15	-0.25
6 h	-0.12	--	0.19	-0.12	-0.24	-0.28
12 h	0.00	0.19	--	0.06	-0.14	0.28
24 h	-0.11	-0.12	0.06	--	0.37	0.64**
48 h	-0.15	-0.24	-0.14	0.37	--	0.55**
72 h	-0.25	-0.28	0.28	0.64**	0.55**	--
Serum BUN, mg/dL						
0 h	--	0.63**	0.30	0.09	0.04	-0.08
6 h	0.63**	--	0.88**	0.57**	0.23	0.11
12 h	0.30	0.88**	--	0.83**	0.52**	0.45*
24 h	0.09	0.57**	0.83**	--	0.75**	0.57**
48 h	0.04	0.23	0.52**	0.75**	--	0.84**
72 h	-0.08	0.11	0.45*	0.57**	0.84**	--
Serum total protein, g/dL						
0 h	--	0.19	0.15	0.18	0.03	-0.10
6 h	0.19	--	0.74**	0.45**	0.57**	0.38*
12 h	0.15	0.74**	--	0.84**	0.90**	0.40
24 h	0.18	0.45**	0.84**	--	0.97**	0.10
48 h	0.03	0.57**	0.90**	0.97**	--	0.14
72 h	-0.10	0.38*	0.40	0.10	0.14	--
Serum globulin, g/dL						
0 h	--	-0.12	-0.23	0.18	0.01	0.16
6 h	-0.12	--	0.64**	0.44**	0.52**	0.21
12 h	-0.23	0.64**	--	0.85**	0.90**	0.40
24 h	0.18	0.44**	0.85**	--	0.98**	0.10
48 h	0.01	0.52**	0.90**	0.98**	--	0.71**
72 h	0.16	0.21	0.40	0.10	0.71**	--

**$P \leq 0.05$; *$P \leq 0.10$.

TABLE 4. Partial correlation of coefficients of postnatal calf sampling time for serum blood urea nitrogen, plasma glucose, serum total protein, and serum globulin concentrations between sampling hours in spring-born calves (Exp. 1 and 2)

	Sampling hour, postnatal age					
Items	0 h	6 h	12 h	24 h	48 h	72 h
Serum glucose, mg/dL						
0 h	--	0.38**	0.40**	0.04	0.12	0.10
6 h	0.38**	--	0.75**	0.43**	0.34**	0.42**
12 h	0.40**	0.75**	--	0.41**	0.39**	0.53**
24 h	0.04	0.43**	0.41**	--	0.56**	0.38**
48 h	0.12	0.34**	0.39**	0.56**	--	0.68**
72 h	0.10	0.42**	0.53**	0.38**	0.68**	--
Serum NEFA, mEq/L[1]						
0 h	--	0.56**	0.10	0.11	0.34*	0.12
6 h	0.56**	--	-0.07	0.23	-0.07	0.06
12 h	0.10	-0.08	--	0.35**	0.53**	-0.08
24 h	0.11	0.23	0.35	--	0.24	-0.08
48 h	0.34*	-0.07	0.53	0.24	--	0.35**
72 h	0.12	0.06	-0.08	-0.08	0.35**	--
Serum BUN, mg/dL						
0 h	--	0.84**	0.47**	0.09	-0.19	-0.30**
6 h	0.84**	--	0.76**	0.39**	0.02	-0.21
12 h	0.47**	0.76**	--	0.76**	0.38**	0.08
24 h	0.09	0.39**	0.76**	--	0.69**	0.36**
48 h	-0.19	0.02	0.38**	0.69**	--	0.69**
72 h	-0.30**	-0.21	0.08	0.36**	0.69**	--
Serum total protein, g/dL						
0 h	--	0.14	0.06	0.08	0.02	0.01
6 h	0.14	--	0.77**	0.67**	0.67**	0.61**
12 h	0.06	0.77**	--	0.90**	0.87**	0.85**
24 h	0.08	0.67**	0.90**	--	0.95**	0.90**
48 h	0.02	0.67**	0.87**	0.95**	--	0.94**
72 h	0.01	0.61**	0.85**	0.90**	0.94**	--
Serum globulin, g/dL						
0 h	--	0.13	0.00	-0.10	-0.05	-0.06
6 h	0.13	--	0.82**	0.67**	0.70**	0.67**
12 h	0.00	0.82**	--	0.90**	0.89**	0.88**
24 h	-0.10	0.67**	0.90**	--	0.97**	0.93**
48 h	-0.05	0.70**	0.89**	0.97**	--	0.94**
72 h	-0.06	0.67**	0.88**	0.93**	0.94**	--

[1]Exp. 2 only.

**$P \leq 0.05$; *$P \leq 0.10$.

Metabolites at 0 h were correlated with few other sampling times, which may be due the changes in metabolites over time and the difference in pre- and postnatal delivery of nutrients. There was a greater number of positive relationships in metabolites from consecutive sampling times and may be due to the similar intake of colostrum or milk after the initial intake of the postnatal nutrients, especially after 24 h of age.

Better knowledge of sampling time differences is necessary to determine the relationship of neonatal metabolic status, vigor, and survival.

These data demonstrate that a consistent sampling time is necessary due to the changes in metabolites over time in neonatal beef calves. To better determine prenatal metabolite concentrations, samples need to be collected prior to colostrum intake, however, the current study suggests metabolite concentrations are not consistent during the first 72 h, therefore sampling time does matter during the neonatal period.

LITERATURE CITED

Larson, J. M. 2016. Metabolic status of late gestation beef cows and neonatal calves. MS Thesis, University of Missouri, Columbia, MO.

Blood oximetry responses of glycerin-supplemented and immune-challenged calves[1]

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ABSTRACT: Immunocompromised calves have been reported to have a greater need for energy. This study evaluated the effects of glycerin supplementation and an endotoxin challenge on blood gas parameters. Crossbred beef steers (n = 24) divided into 2 blocks based on initial BW (block 1 = 214 ± 4.4 kg; block 2 = 193 ± 4.5 kg) were used in a randomized complete block design. Treatments were a 2 × 2 factorial arrangement of 0 or 25 g/L (-GLY vs. +GLY) of glycerin in drinking water, and 0 or 3 μg kg^{-1} (-LPS vs. +LPS) of bacterial lipopolysaccharide (LPS) administered via a saline based subcutaneous injection. The experiment consisted of a 7-d adaptation period followed by a 12-h collection period. On d 8, the LPS treatments were administered. Respiration rates were measured and blood samples collected at 0, 2, 4, 8, and 12 h relative to the LPS administration. All data were statistically analyzed using mixed models and repeated measures. An LPS × GLY interaction (P = 0.05) occurred for blood pH; pH was not different for +GLY and -GLY calves when challenged with LPS, but tended to be lower for +GLY than -GLY when not immune challenged. An LPS × h interaction occurred ($P \leq 0.01$) for blood pH and pO_2, and concentrations of blood glucose and lactate. Blood pH was greater for +LPS than -LPS at 2, 4, 8, and 12 h after LPS injection. Blood pO_2 was lower for +LPS than -LPS at 2 and 4 h, and not different at 8 and 12 h. Blood glucose was lower for steers injected with +LPS than -LPS at 4 and 8 h, and not different between LPS treatments at 12 h. Blood lactate concentrations were greater for +LPS than –LPS steers at 2, 4, 8, and 12 h after the LPS injection. Blood pO_2 was greater (GLY effect; P = 0.01) for +GLY than -GLY steers. In conclusion, respiratory function is compromised for endotoxin-challenged steers and results in lower blood oximetry values. Crude glycerin supplementation did not alleviate the effect of an endotoxin challenge on respiratory function. Also, glycerin supplementation did not appear to improve energy status of immune-challenged steers based on the inability to alleviate the hypoglycemic effects associated with an endotoxin challenge.

Key words: blood gas, glycerin, health, steer
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INTRODUCTION

Bovine respiratory disease (**BRD**) is the greatest health concern facing the cattle feeding industry accounting for more than $692-million in economic losses annually (NASS, 2006). The anticipated restriction on antibiotic use in food producing animals could amplify these losses. Early detection of respiratory disease could mitigate these losses and minimize antibiotic use. Blood oximetry values, used in human medicine to diagnose pneumonia, have been correlated to BRD (Oosthuysen et al., 2016) and arterial hypoxia (Gebeyehu, 2010) in cattle, which imply the possible use for early detection of BRD.

Calves exposed to gram-negative bacterial lipopolysaccharide (**LPS**) exhibit physiological and immune responses resembling those suffering from respiratory disease. Such responses include increased rectal temperatures, serum cortisol, cytokine, and haptoglobin concentrations, and decreased serum glucose concentrations (Waggoner et al., 2009a,b). Calves suffering from BRD have been reported to also have decreased plasma glucose values (Montgomery et al., 2009; Oosthuysen et al., 2016),

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suggesting a greater need for energy to support the immune response. These effects are exacerbated by suppressed feed intakes in morbid animals (Preston, 2007).

Crude glycerin, a byproduct of the biodiesel industry, has become an attractive energy supplement in ruminant diets due to its glucogenic properties (Lee et al., 2011). However, stressed morbid calves reluctant to consume feed limits the effectiveness of glycerin in feed. Nevertheless, glycerin dissolved in drinking water could serve as an alternative supply of energy for anorexic calves. We hypothesized that supplementing calves with crude glycerin in their drinking water could alleviate the effects of an endotoxin challenge on respiratory function as observed through blood gas analysis. Therefore, the objective of the study was to evaluate blood gas parameters of endotoxin-challenged and glycerin-supplemented calves.

MATERIALS AND METHODS

Animal, Facilities, and Diet

The New Mexico State University Institutional Animal Care and Used Committee approved all animal procedures. This experiment was conducted in an evaporatively cooled (22.6 ± 2.03°C) animal metabolism facility using 24 ruminally-cannulated crossbred beef steers (203 ± 3.8 kg BW). Animals were housed in individual tie-stalls each padded with 2.54 cm thick rubber mats. Animals were fed a diet (Table 1) at 1.7% of BW (DM basis) in equal proportions twice daily at 0700 and 1900 h throughout the experiment. This experiment was a companion study to Carey et al. (2017) and Lopez et al. (2017).

Experimental Design and Treatments

The experiment was a randomized complete block design with 24 cannulated steers divided into 2 blocks based on initial BW (block 1= 214 ± 4.4 kg; block 2 = 193 ± 4.5 kg). Steers within each block were randomly assigned to a 2 × 2 factorial arrangement of treatments. These treatments were no glycerin (**-GLY**) or 25 g/L of glycerin (**+GLY**) in drinking water factorially arranged with a subcutaneous injection of no LPS (**-LPS**) or 3 µg (**+LPS)** of *Escherichia coli* O55:B5 (Sigma Chem. Co., St. Louis, MO) per kg of BW dissolved in 2 mL of sterile saline. The experiment consisted of a 7-d adaptation period followed by a 12-h collection period. On d 6, indwelling catheters (J-457A; Jorgensen Laboratories, Loveland, CO) were placed into the jugular veins of steers. On d 8 at 0900 h (2 h after the 0700 h feeding), LPS treatments were administered. Steers had ad libitum access to their individual water treatments throughout the experiment.

Table 1. Nutrient composition of diet

Nutrient[1]	Diet
CP, % of DM	15.11
NE_m, Mcal/kg DM	1.74
NE_g, Mcal/kg DM	1.12

[1]Analyzed by SDK Laboratories (Hutchinson, KS).

[2]ME, Mcal/kg = 0.01×(81.81-0.48×%ADF)×4.409×0.82; NE_m, Mcal/kg = 1.37×ME-0.138×ME^2+0.0105×ME^3-1.12; NE_g, Mcal/kg = 1.42×ME-0.174×ME^2+0.0122×ME^3-1.65 (NRC, 2000).

The crude glycerin contained 85% glycerol and less than 0.01% methanol on an as-fed basis (analyzed by SDK Laboratories, Hutchinson, KS).

Sample Collections

On d 8, respiration rates were measured and blood samples collected at 0, 2, 4, 8, and 12 h relative to the LPS administration. Respiration rates were measured using a stopwatch to count the number of breaths per minute through visual observation of the rhythmic movement of the lumbar region of the animal. Venous blood samples were collected via the jugular catheter into 2-mL heparin syringes (Pico 50; Radiometer Medical, Denmark) with a unique TIPCAP to minimize oxygen contamination and blood clotting before the blood samples were aspirated into a benchtop blood gas analyzer (ABL815 FLEX; Radiometer America Inc., Westlake, OH). Blood parameters measured by the blood gas analyzer included pH, the partial pressure of oxygen (**pO_2**), and concentrations of blood glucose and lactate.

Statistical Analysis

Respiration rate and blood parameters were statistically analyzed as a randomized complete block design using mixed models (SAS Inst. Inc., Cary, NC). Data collection occurred over two periods (12 animals per period) due to metabolism facility constraints. Animals served as experimental unit blocked into two groups based on the two periods and initial BW. The statistical model included the effects of GLY, LPS, hour, and all possible interactions. Hour was added as repeated measure to the model with autoregressive order-one as covariance structure. Block and steer were random. Differences among treatments were considered significant when $P \leq 0.05$.

RESULTS

No LPS × GLY × h interactions occurred ($P \geq 0.09$) for all response variables measured. Also, no LPS × GLY

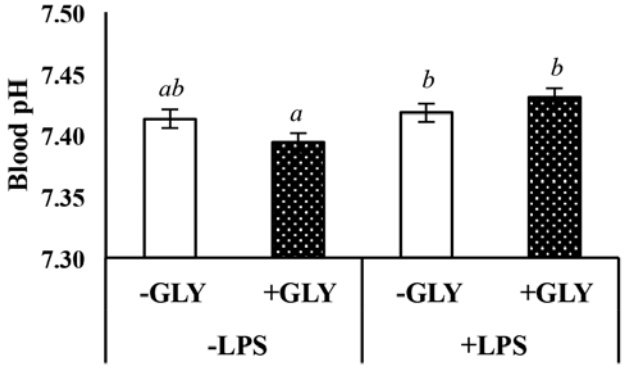

Figure 1. Blood pH of steers in response to a subcutaneous lipopolysaccharide injection of 0 or 3 µg (-LPS vs. +LPS) per kg of BW and glycerin added to drinking water at 0 of 25 g/L (-GLY vs. +GLY). Effects were: LPS × GLY × h ($P = 0.09$), LPS × GLY ($P = 0.05$), LPS × h ($P = 0.01$), GLY × h ($P = 0.20$), LPS ($P = 0.01$), and GLY ($P = 0.66$).

interactions occurred ($P \geq 0.13$) for respiratory rates, blood oximetry values, or blood lactate and glucose concentrations. An LPS × GLY interaction ($P = 0.05$) occurred for blood pH; blood pH was not different for -GLY (7.42) and +GLY (7.43) calves when challenged with LPS, but tended to be lower for +GLY (7.39) than -GLY (7.41) when not immune challenged (Fig. 1).

An LPS × h interaction occurred ($P \leq 0.01$) for blood pH and pO_2, and concentrations of glucose and lactate (Fig. 2). Blood pH increased from 0 to 4 h, and were greater for +LPS than -LPS at 2, 4 (peak), 8, and 12 h after LPS injection. Blood pO_2 decreased for +LPS (and increased for -LPS) from 0 to 2 h, were lower for +LPS than -LPS at 2 (nadir) and 4 h, and not different at 8 and 12 h. Blood glucose increased from 0 to 2 h for both +LPS and –LPS steers, then decreased from 2 to 4 h for +LPS steers and were lower for steers injected with +LPS than -LPS at 4 and 8 h, and not different between LPS treatments at 12 h. Blood lactate concentrations of +LPS steers increased from 0 to 2 h (peak), and were greater for +LPS than –LPS steers at 2, 4, 8, and 12 h after the LPS injection. No GLY × h interaction occurred ($P = 0.81$) for blood pO_2 (Fig. 3), but pO_2 was greater ($P = 0.01$) for +GLY (36.3 mm/Hg) than -GLY (34.5 mm/Hg).

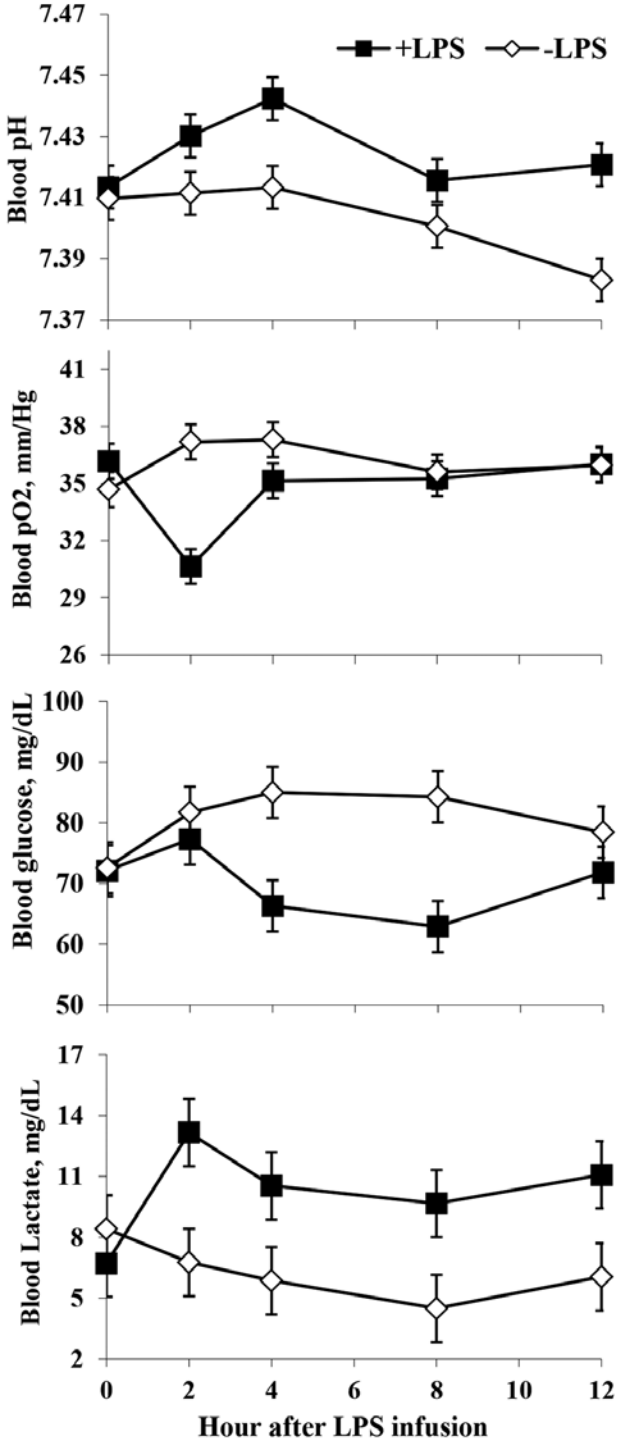

Figure 2. Blood pH, blood pO_2, blood glucose, and blood lactate concentrations of steers in response to a 2 mL subcutaneous bacterial lipopolysaccharide injection to provide 0 or 3 µg (-LPS vs +LPS) per kg of BW. Effects of LPS × h ($P \leq 0.01$).

DISCUSSION

Pneumonia can be described as an inflammatory condition of the lung in which some or all alveoli become filled with fluid and blood cells (Guyton, 1991), compromising the ability for gas exchange. Therefore, we hypothesized that the inflammatory response caused by an LPS challenge will compromise respiratory function which would be evident in lower blood oximetry values. The lower pO_2 at 2 and 4 h for LPS calves suggest that the endotoxin challenge did compromise respiratory function as expected. Due to the comprised ability for gas exchange, blood pCO_2 expiration in the alveoli is expected to decrease resulting in a buildup of blood pCO_2.

Carbonic acid formation when CO_2 interacts with water as it enters the blood causes the blood environment to become more acidic. However, blood pH and oximetry values are carefully regulated by the direct

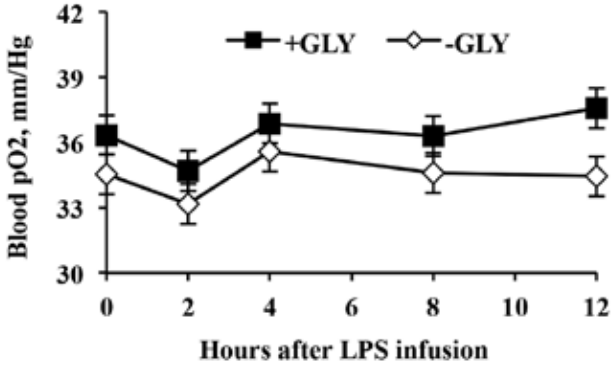

Figure 3. Steer blood partial pressure of oxygen (pO_2) in response to 0 or 25 g/L of crude glycerin (-GLY vs. +GLY) in the drinking water. Effects were GLY × h ($P = 0.81$), GLY ($P = 0.01$).

chemical control through the effect of CO_2 and H^+ ions on the respiratory center (Guyton, 1991). The chemo sensitive area of the respiratory center is excited by increasing CO_2 and H^+ ions and elicits an increased respiration rate resulting in more CO_2 being expelled (Guyton, 1991). The increased blood pH observed for immune-challenged calves could be explained by a shift of the oxygen-hemoglobin dissociation curve to increase the affinity of hemoglobin for oxygen by increasing the respiration rate and consequently the blood pH. It is plausible that the nature of monitoring respiration rates (visually with a stopwatch) limited the ability to detect an increase in LPS steers.

Glycerin in the rumen is rapidly fermented to propionate (Lee et al., 2011; Lopez et al., 2017), and due the glucogenic properties of propionate we hypothesized that crude glycerin supplementation would improve the energy status of calves. In contrast to our hypothesis, blood glucose was not different among glycerin treatments. Oosthuysen et al. (2016) reported a positive correlation between blood glucose concentrations and pO_2, suggesting an improved energetic status to be correlated with greater respiratory function. Although +GLY calves did not express elevated serum glucose, a similar increase in blood pO_2 was observed for glycerin supplemented calves.

As part of the stress response to an endotoxin challenge, large amount of stress hormones are secreted to signal the release of energy for utilization by an activated immune system (Grandin, 1999). A decrease in blood glucose concentrations for +LPS steers is consistent with the findings of Samuelson et al. (2014). These authors reported a brief increase in serum insulin concentration accompanying a brief increase in serum glucose, followed by a hypoglycemic state after LPS infusion. Therefore, the lowered blood glucose concentrations observed at 4 and 8 h for +LPS steers could be attributed to the effects of insulin. Calves exposed to an LPS challenge express many innate immune responses such as increased rectal temperatures, serum cortisol, and cytokine secretions (Waggoner et al., 2009a,b). These responses involve an energetic cost and could contribute to the lowered blood glucose concentrations observed in +LPS calves.

Endotoxin-challenged steers have increased glucocorticoid secretions (Samuelson et al., 2014) which result in rapid glycolysis and lactate production (Shaw and Tume, 1992). Lactate is the end-product of anaerobic glucose metabolism. We hypothesized that blood lactate levels would be greater for +LPS steers due to effect of LPS on respiratory function and glucocorticoid secretions. The elevated serum lactate concentrations accompanied by a lower pO_2 of +LPS steers is in support of our hypothesis and possibly a result of increased anaerobic metabolism.

In summary, respiratory function is compromised for endotoxin-challenged steers and results in lower blood oximetry values. Crude glycerin supplementation in the drinking water did not alleviate the effect of an endotoxin challenge on respiratory function. Also, glycerin supplementation did not appear to improve energy status of immune-challenged steers based on the inability to alleviate the hypoglycemic effects associated with an endotoxin challenge.

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Effects of fish meal supplementation on forage intake and metabolizable protein of beef cows grazing winter wheat pasture[1]

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ABSTRACT: Eight ruminally and duodenally cannulated, angus-crossbred cows (665 ± 20.6 kg) grazing winter wheat pasture (WWP) were used in a complete randomized design to evaluate the effects of fish meal (FM) supplementation on forage intake, characteristics of digestion and metabolizable protein of cattle grazing WWP. The experiment was conducted during 13 d from March 7 through March 19, 2016. The first 10 d were used for adaptation to WWP grazing and FM supplementation, and the last 3 d for sample collection. Cows grazed in a single WWP. Treatments consisted of cows (4 cows per treatment) grazing in a single pasture and supplemented with FM to provide: control, no RUP (CON), or supplemented at a level calculated to supply 10% of the forage CP intake as RUP (FM). Supplemental FM was placed directly into the rumen cannula once daily at 0700 h. Forage DM, NDF, CP, total OM intake, OM intake expressed as g/kg of BW were not affected ($P \geq 0.44$) by FM supplementation. Also, supplemental FM had no effects on microbial protein efficiency ($P = 0.34$), total CP flowing to duodenum (MP; $P = 0.70$), microbial protein synthesis ($P = 0.70$) or feed protein bypassing rumen fermentation ($P = 0.72$). Moreover, true ruminal, or total tract digestibility of OM, NDF, and CP were not affected ($P \geq 0.38$) by FM supplementation. Ruminal pH (6.37, and 6.43 ± 0.09 for CON and FM, respectively), total VFA production (50.2, and 49.7 ± 0.1.74 m*M* for CON and FM, respectively), acetate (74.4, and 74.6 ± 0.77 mol/100 mol for CON and FM, respectively), propionate (16.7, and 16.1 ± 0.62 mol/100 mol for CON and FM, respectively), and acetate/propionate ratio (4.49 and 4.69 ± 0.22 for CON and FM, respectively) were not affected by FM supplementation ($P \geq 0.49$). Although FM supplementation did not decrease forage intake, it failed to improve MP of cows grazing WWP. The results indicate that improvements in performance of cattle grazing WWP should not be expected with supplementing FM.

Key words: fish meal, MP, RUP, winter wheat pasture
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INTRODUCTION

Winter wheat pasture (*Triticum aestivum*) is a high quality forage with DM digestibility over 70%, CP content over 20%, and ADF content under 30% (Mader and Horn, 1986; Branine and Galyean, 1990; Vogel et al., 1989). However, CP in situ effective degradability of winter wheat pasture (WWP) has been estimated to be over 90% (Chabot et al. 2008). Therefore, the CP from WWP that reach the small intestine for absorption may be limited (Beever, 1984). This suggests that most of the protein absorbed by cattle grazing WWP is from microbial origin.

Synthesis of microbial protein depends of OM available for ruminal fermentation and the presence of N-containing compounds (Hespell, 1979). The low fiber or NDF content of WWP might limit the potential of cattle grazing WWP to maximize microbial synthesis. Therefore, it is hypothesized that supplementation with RUP protein might increase the quantity of protein that reach the small intestine for absorption (MP). The RUP content of fish meal (FM) is approximately 60% (NRC, 1996), with AA profile similar to that required for bovine growth and milk production (Tamminga, 1982). However, the effects of FM supplementation on MP of cattle

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grazing WWP have not been elucidated. The objective of this experiment was to evaluate the effects of FM supplementation on forage intake, characteristics of digestion and metabolizable protein of cattle grazing WWP.

MATERIALS AND METHODS

All procedures and experimental protocols were approved by the New Mexico State University Institute Animal Care and Use Committee.

Eight mature Angus crossbred cows (665 ± 20.6 kg of BW) fitted with duodenal and ruminal cannulas were used in a complete randomized design. The study was conducted during 13 d from March 7 through March 19. The first 10 d were used for adaptation to WWP grazing and FM supplementation, and the last 3 d for sample collection. The cows were randomly assigned to 1 of 2 treatments (4 cows per treatment): 1) control, no FM (**CON**), and FM supplemented at a level calculated to supply 10% of forage CP as RUP (FM). Cows grazed a single beardless winter irrigated wheat pasture (Weathermaster 135; *Triticum aestivum*; Weathermaster 135). At the beginning of the experimental period the plants had complete the stem elongation and by the end of the experimental period the plants were at in boot stage of development. Every day at 0700, cows were gathered into a holding pen, tied to a fence post with 2-m long halter, and their allotted supplement was place into their rumen through their rumen cannula.

Chyme reaching the duodenum and fecal output were estimated using chromic oxide as an undigestible marker. Gelatin capsules containing 8 g of chromic oxide were dosed ruminally twice daily (0700 and 1900) on d 6 through 13 of the experiment. Eight fecal samples from rectal grabs and 8 duodenal samples from duodenal cannulas were collected in a 3-d period to represent 1 every 3 h in a 24-h period. Duodenal and fecal samples were collected from all cows as follows: d 11) 700, 1600 and 2200 h; d 12) 100, 1300, and 1900 h; and d 13) 400, and 1000 h. Individual samples consisted of approximately 300 mL of duodenal chyme and 200 g (wet basis) of fecal material. Samples from each cow were composited for analysis. Ruminal fluid samples were collected on d 12 of the experimental period at 0 (before dosing), 2, 4, 6, 8, 10 and 12, h after dosing. Ruminal fluid pH was determined immediately after collection, and the samples were then acidified with 7.2 *N* NH_2SO_4 at a rate of 1 mL/25 mL of ruminal fluid and cooled (4 °C) for later analysis of ammonia, and VFA. At 1100 h on d 13 of the experimental period, 5 L of ruminal fluid was obtained and mixed with 5 L of saline solution (0.9% NaCl, wt/vol) for isolation of bacterial cells (Zinn and Owens, 1986). Ruminal fluid/saline solution mixture was cooled (4 °C) for later bacterial isolation. Ruminal fluid/saline mixture was centrifuged at 1,000 × *g* for 10 min to remove feed particle and protozoa from the supernatant. Supernatant was centrifuged at 27,000 × *g* for 20 min to separate bacteria from the supernatant, and then the isolated bacteria was resuspended in saline and centrifuged at 27,000 × *g* for 20 min. The final isolated bacteria were collected and freeze-dried (-50°C) for 96 h.

Two of the 8 cannulated cows grazing the WWP were ruminally evacuated on d 13 after the last sample collection of the experimental at 1200 h. Digesta was placed at in plastics bags lining a 133-L plastic container. Evacuated cows were allowed to graze WWP for 1 h. Then cows were regathered and masticate samples were collected and dried in a force air oven (55°C) to a constant weight, and ground in a Wiley mill (2-mm screen; Wiley mill model 4, Thomas Scientific, Swedesboro, NJ), and composited on an equal weight basis.

Laboratory Analysis. Duodenal, fecal and bacterial samples were thawed, mixed, subsampled, and dried in a freeze dryer (-50°C) for 96 h, and ground in a Wiley mill (2-mm screen). Duodenal, fecal, masticate, and supplement samples were analyzed for, DM, ash, and CP (Methods 930.15, 942.05, and 990.02, respectively; AOAC, 1997). Samples were analyzed for NDF according to Van Soest et al. (1991) using an Ankom 200 fiber analyzer (Ankom CO, Fairport, NY). Concentration of Cr was determined in duodenal and fecal samples (Hill and Anderson, 1958). Duodenal samples were analyzed for purines (Zinn and Owens, 1986). Isolated ruminal bacteria were analyzed for DM, N, ash, and purines as previously described. Ruminal fluid samples were centrifuged at 27,000 × g for 20 min and analyzed for VFA (Goetsch and Galyean, 1983). Indigestibility of masticate and FM were estimated with

Calculations. Daily fecal DM output and chyme DM leaving the abomasum were calculated using fecal and duodenal Cr concentration, respectively. Fecal DM output was calculated by dividing Cr dose by fecal Cr concentration. Similarly, chyme DM leaving the abomasum was calculated by dividing Cr dose by duodenal chyme Cr concentration. Supplement fecal DM output was calculated by multiplying supplement intake by supplement DM indigestibility, and forage fecal DM output was calculated by subtracting supplement fecal DM output from fecal DM output. Forage DM intake was calculated as forage fecal output divided by forage in vitro indigestibility. Therefore, total tract digestion of forage DM was not different. The small contribution of FM indigestibility caused enough change that *P*-value was calculated to be 0.01. That *P*-value

does not imply effects of FM on total tract DM digestibility. Changes in forage intake are expected when treatment affects total tract DM digestibility when the methodology used in the present study is used to estimate forage intake of grazing cattle. Microbial OM and N leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter fermented in the rumen was considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and microbial OM reaching the duodenum. Feed N reaching the small intestine was considered equal to total N leaving the abomasum minus NH_3-N and microbial N and, thus, includes endogenous N additions. Microbial N efficiency was calculated as g of duodenal microbial N per kg of OM fermented in the rumen. Nutrient intake and nutrient digestibility were calculate based on analyzed chemical composition of collected wheat pasture masticates and supplemental FM. Masticate analyzed composition was 18.03% ash, 13.67% CP, and 56.24% CP. While FM analyzed composition was 23.17% ash, 65.70% CP, and 20.32% NDF.

Statistical Analysis. Data were analyzed as a completely randomized design with GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effect of FM supplementation. Effects were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Effects of FM supplementation on forage intake and characteristics of digestion of cows grazing WWP are shown in Table 1. Daily FM supplementation was 246.2 ± 6.2 g and was it calculated to supply 147.6 g of RUP which also was determined to be 10% of the predicted CP intake from forage. However, forage consumption was greater than estimated. Therefore, the RUP provided by supplemental FM was only 8.6% of the CP consumed from forage. Forage DM, NDF, CP, total OM intake, OM intake expressed as g/kg of BW were not affected ($P \geq 0.44$) by FM supplementation. Although the high solubility of CP of wheat forage has been known for decades (Horn et al., 2005), limited information exist on the effects of RUP supplementation on wheat forage intake. Similar DMI of freshly harvest wheat forage has been reported for lambs supplemented with either energy or high RUP supplements (Phillips et al., 1995). Because WWP is not deficient in RDP (Burroughs et al., 1974), no effects of FM supplementation on forage intake were expected in the present experiment. Supplemental FM had no effects on microbial protein efficiency ($P \geq 0.34$), total CP flowing to duodenum (MP; $P = 0.70$), microbial protein synthesis ($P = 0.70$) or feed protein bypassing rumen fermentation ($P = 0.72$). True ruminal, or total tract digestibility of OM, NDF, and CP were not affected ($P \geq 0.38$) were not affected by FM supplementation. Ruminal pH (6.37, and 6.43 ± 0.09 for CON and FM, respectively), total VFA production (50.2, and 49.7 ± 0.1.74 m*M* for CON and FM, respectively), acetate (74.4, and 74.6 ±

TABLE 1. Effects of fish meal supplementation on forage intake and characteristics of digestion of beef cows grazing winter wheat pasture

	Fish meal supplementation, g/d			
Item	0	375	SE	*P*-value
DM intake, g/d				
Forage	12,658	12,564	1,454.7	0.96
Fish meal	-	374.7	-	-
Total	12,658	12,939	1,456.9	0.89
OM intake, g/d				
Forage	10,375	10,298	1,192.4	0.96
Fish meal	-	287.9	-	-
Total	10,375	10,587	1,194.1	0.96
Total OM intake g/kg of BW	15.5	16.0	1.7	0.85
CP intake, g/d				
Forage	1,730	1,717	198.86	0.96
Fish meal	-	246.2	-	-
Total	1,730	1,964	200.4	0.44
NDF intake, g/d				
Forage	7,118	7,066	818.2	0.96
Fish meal	-	76.1	-	-
Total	7,118	7,142	818.6	0.98
Flow to Duodenum, g/d				
DM	6,946	7,026	1,058.8	0.95
OM	4,926	4,969	778.7	0.97
NDF	1,886	2,051	374.1	0.76
CP	1,778	1,924	260.2	0.70
Microbial protein	906	981	133.3	0.70
Feed protein	872	943	132.1	0.72
Microbial protein efficiency[1]	29.5	32.4	2.02	0.34
True ruminal digestion, %				
DM	70.7	72.1	3.94	0.80
OM	65.7	67.2	3.19	0.75
CP	49.8	52.2	3.47	0.65
Fecal excretion, g/d				
OM	1,886	1,978	288.5	0.78
CP	485	530	65.5	0.65
NDF	1,565	1,572	181.6	0.97
Total tract digestion, %				
DM	75.4	75.3	0.005	0.01
OM	81.7	81.4	0.43	0.62
CP	71.8	73.3	1.42	0.48
NDF	78.0	77.9	0.72	0.89
Total tract digestion, g				
DM	9,544	9,746	1,098	0.90
OM	8,488	8,608	971	0.93
CP	1,245	1,434	142.8	0.38
NDF	5,553	5,569	647.2	0.98

[1]Microbial protein efficiency = Duodenal microbial N, g.kg $^{-1}$ OM truly fermented in the rumen.

0.77 mol/100 mol for CON and FM, respectively), propionate (16.7, and 16.1 ± 0.62 mol/100 mol for CON and FM, respectively), and acetate/propionate ratio (4.49 and 4.69 ± 0.22 for CON and FM, respectively) were not affected by FM supplementation ($P \geq 0.49$). Synthesis of microbial protein, digestibility of OM and NDF, and rumen fermentation characteristics were expected to not be affected by FM supplementation because WWP has more than adequate RDP (Burroughs et al., 1974). However, FM supplementation was expected to increase the protein from feed origin flowing to duodenum (RUP from WWP and FM). As a result of increasing RUP, it was expected to increase the total CP flowing to duodenum (MP). Previous research showed that RUP supplementation as compared with energy supplementation (control) improved ADG of steers grazing actively growing smooth brome grass above (Anderson, et al., 1988), but not to steers grazing wheat pasture (Vogel et al., 1989; Smith et al., 1989). Results from this experiment suggests that previous RUP supplementation experiments of cattle grazing wheat pasture failed to improve ADG above that on energy supplemented cattle because such supplementation failed to improve MP status.

IMPLICATIONS

Results from this experiment imply that FM supplementation as a source of RUP does not affected MP of cattle grazing WWP, and therefore it is not expected to improve productive performance.

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Substituting ground juniper for ground alfalfa hay in steer feedlot diets: Growth performance and blood serum characteristics[1]

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ABSTRACT: A randomized design study with two feeding periods was used to evaluate effects of substituting ground juniper for ground alfalfa hay in steer feedlot diets on growth performance and blood serum characteristics. Steers (n = 44; initial BW = 329 kg) were fed in a Calan gate system (4 feeders/pen; 8 pens) for 112 d. During Period 1, steers were fed a 70% concentrate diet from d 0 to 69 d that differed only by roughage source; juniper replaced 0, 33, 66 or 100% of the alfalfa (0JUN, 33JUN, 66JUN, or 100JUN, respectively). During Period 2 (d 70 to 112), all steers were transitioned onto a common alfalfa-based 90% concentrate finishing diet. Treatment × day interactions ($P < 0.001$) were observed for steer DMI, ADG, and G:F. As percentage of juniper increased in the diet, daily DMI linearly decreased ($P < 0.01$) on d 14, 28, 42, 56, and 70, but steer DMI was similar during Period 2. As percentage of juniper increased in the diet, ADG linearly decreased ($P < 0.01$) on d 14 and 42, tended to linearly decrease ($P = 0.09$) on d 28, and tended to quadratically decrease ($P = 0.07$) on d 56. No differences in steer ADG was observed on d 70 or 84. However, as percentage of juniper increased in the diet, ADG linearly increased ($P < 0.001$) and tended to quadratically increase ($P = 0.07$) on d 98 and 112, respectively. Negative linearly trends ($P < 0.01$) were observed for steer BW at each weigh date, resulting final BW being less ($P < 0.05$) for steers fed 66JUN and 100JUN vs. steers fed 0JUN, at the end of each period (on d 70 and 112). As percentage of juniper increased in the diet, G:F linearly decreased ($P < 0.001$) on d 14, quadratically decreased ($P = 0.03$) on d 56, and linearly and quadratically increased ($P < 0.03$) on d 98 and 112, respectively. Results suggested that replacing ground alfalfa with ground juniper did not negatively affect steer health as assessed by daily visual appraisal and blood serum chemistry and that the inclusion of 10% juniper (33JUN) should be considered economically viable.

Key words: feedlot, juniper, secondary metabolites, steers

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INTRODUCTION

Roughage ingredients are the most expensive components of beef cattle feedlot diets on the basis of cost/kg of energy or protein. All roughage and grain feed ingredients are seasonal, thus experience yearly price fluctuations. This is especially true during drought conditions, if the ingredient is even available. However, ground woody products do not experience seasonal price fluctuations or availability, have an extensive history of being effective roughage ingredients (Maynard, 1920; Marion et al., 1959, and require no inputs from man to grow. *Juniperus pinchotii* and *J. ashei* are common encroaching woody plants in Texas that are significantly reducing natural resources (Scholes and Archer, 1997). Removing and processing *Juniperus* spp. into a roughage ingredient for ruminant livestock would not only subsidize woody plant management efforts, but would also provide a cost-competitive feed ingredient while synergistically increasing the potential for understory herbaceous growth (Coultrap et al., 2008).

Even though nutrient and digestive characteristics of ground *Juniperus* spp. is similar to many traditional low-quality roughage ingredients (Stewart, et al., 2015; NRC, 2016), maximum inclusion rate in beef cattle feedlot diets that does not

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negatively affect growth performance, animal health, or cost/kg of BW gain is unknown. Thus, the objective was to evaluate effects of substituting *Juniperus* spp. for alfalfa hay in steer feedlot diets on growth performance and blood serum characteristics. The hypothesis was that: (1) DMI and ADG would linearly decrease as juniper incrementally replaced alfalfa; (2) animal health would not be negatively affected; and (3) ground juniper, at some concentration, would enhance G:F and reduce cost/kg of BW gain.

MATERIAL AND METHODS

Animals and Management

Experimental protocol was approved by the Texas A&M University Institutional Animal Care and Use Committee (#2016-009A). In a randomized design, steers (n = 44; initial BW = 329 kg) were assigned to a treatment diet and placed into a Calan gate system (4 feeders/pen; 8 pens). A 14-day period was used to train the steers to the Calan gates. From d 0 to 69 d (Period 1), steers were fed a 70% concentrate diet that differed only by roughage source (Table 1); juniper replaced 0, 33, 66 or 100% of the alfalfa (4 treatments). Beginning on d 70, all steers were transitioned onto a common alfalfa-based 90% concentrate finishing diet, which was fed until the end of the trial; d 112 (Period 2).

Amount of feed offered was adjusted as needed to achieve approximately 0.45 kg/head of refusal. Orts were weighed and subsampled every 7 days. Steers were weighed and blood collected on d 0 and then every 14 d throughout the trial. Steer ADG and average daily DMI were determined between days that BW was recorded; G:F was calculated between weigh days by dividing ADG by average daily DMI. In addition, cost/metric ton of delivered feed (DM basis) was calculated by using the actual price of purchased ingredients. Delivered price of juniper (US $110/dry t) was based on estimated processing costs, after consulting with juniper processing specialists. Cost per kg of diet was divided by kg of BW gain to determine cost of feed per kilogram of BW gain.

TABLE 1. Treatment diet composition and cost of feed

	Diet[1]				
	Period 1				Period 2
Item[2]	0 JUN	33 JUN	66 JUN	100 JUN	0 JUN
Alfalfa hay	30	20	10	0	10
Juniper	0	10	20	30	0
Rolled corn	38	38	38	38	56
DDGS	24	24	24	24	26
Molasses, cane	5.5	5.5	5.5	5.5	5.5
Mineral premix	2.5	2.5	2.5	2.5	2.5
Nutrient Composition, %					
CP,	14.5	13.7	12.4	11.0	15.4
NDF	31.6	33.6	38.4	34.2	18.4
ADF	21.9	24.2	26.9	24.9	.
Lignin	6	7.6	10.3	10.5	2.6
TDN	71	68	64	66	82.2
tIVDMD	84	80	73	74	.
NDFD	49	41	30	25	.
Cost/t	$192	$187	$182	$177	$190

[1]During Period 1, steers were fed a 70% concentrate diet from d 0 to 69 d that differed only by roughage source; juniper replaced 0, 33, 66 or 100% of the alfalfa (0JUN, 33JUN, 66JUN, or 100JUN, respectively). During Period 2 (d 70 to 112), all steers were transitioned onto a common alfalfa-based 90% concentrate finishing diet. The diet fed during Period 2 has yet to be analyzed; thus, reported values were calculated using the Beef Cattle Nutrient Requirements Model (Version 1.0.37.1; NRC, 2016).

[2]DDGS = corn dried distillers grains with solubles. Cost/t = cost of delivered feed/metric ton (DM basis), calculated using the actual price of purchased ingredients. Delivered price of juniper ($110/dry t) was based on estimated processing costs. True 48-h IVDMD (tIVDMD) and NDF digestibility (NDFD, % of NDF) used cattle ruminal fluid.

Sample Collection and Measurements

Feed processing, feed collection, and analysis. The alfalfa hay was ground and the entire above ground biomass from mature *Juniperus ashei* and *J. pinchotii* trees were cut, windrowed, and allowed to air-dry in the pasture for approximately 4 months. Trees were ground (Rotochopper, Inc., Model MC266, St. Martin, MN) and then hammermilled (Bliss 4440, Ponca City, Oklahoma) to pass a 4.76-mm sieve and stored under cover. During Period 1, ground alfalfa, ground juniper, and each treatment diet were sampled three times, combined into 1 sample per ingredient or diet, and evaluated for chemical composition and digestibility (Table 1). Individual ingredients and the common alfalfa-based diet were also collected during Period 2, but have yet to be analyzed; thus, values calculated using Beef Cattle Nutrient Requirements Model (Version 1.0.37.1; NRC, 2016).

Sub-samples were dried to constant weight in a forced-air oven at 105°C to determine percentage of DM. Additional sub-samples were dried at 50°C in a forced-air oven for 48 h, ground through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA), and stored at –20°C. These samples were analyzed for nitrogen by a standard method (method 990.03; AOAC, 2006); CP calculated as 6.25 × N. The NDF and ADF were analyzed according to procedures of Van Soest et al. (1991), which were modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY) using α-amylase and Na sulfite. Lignin was analyzed by a standard method (method 973.18; AOAC, 2006). Crude fat was analyzed by a standard ether extraction method (method 2003.05; AOAC, 2006). Ash was

TABLE 3. Effects of substituting ground juniper for ground alfalfa hay in steer feedlot diets on growth performance

	Diet[1]					*P*-value[2]			
Item[3]	0JUN	33JUN	66JUN	100JUN	SEM[4]	T	Day	T × D	Contrast
DMI, kg/d						0.02	< 0.001	< 0.001	T×D, linear; < 0.001
d 14	9.45	9.21	7.67	5.72	0.56	< 0.001			linear; < 0.001
d 28	10.52	10.10	8.33	7.29	0.50	< 0.001			linear; < 0.001
d 42	11.97	11.68	10.38	8.60	0.56	< 0.001			linear; < 0.001
d 56	12.23	11.73	10.44	9.28	0.62	0.008			linear; < 0.001
d 70	11.24	11.90	9.92	9.03	0.64	0.01			linear; 0.005
d 84	11.17	11.17	10.44	10.21	0.52	0.43			
d 98	11.46	11.84	11.31	12.66	0.65	0.47			
d 112	11.26	11.15	10.25	11.67	0.56	0.34			
ADG, kg						< 0.001	< 0.001	< 0.001	T×D, quadratic; 0.04
d 14	2.85	2.31	1.67	0.31	0.31	< 0.001			linear; < 0.001
d 28	0.98	1.06	0.73	0.53	0.22	0.30			linear; 0.09
d 42	2.03	1.90	1.90	1.11	0.21	0.01			linear; 0.005
d 56	1.10	0.86	0.58	0.97	0.17	0.19			quadratic; 0.07
d 70	1.05	1.24	0.99	0.66	0.19	0.22			
d 84	2.08	1.85	1.86	2.06	0.21	0.80			
d 98	1.02	1.46	1.44	1.89	0.17	0.007			linear; < 0.001
d 112	1.24	1.57	1.26	0.77	0.22	0.10			quadratic; 0.07
G:F, kg/kg						0.002	< 0.001	< 0.001	T×D, quadratic; 0.02
d 14	0.30	0.25	0.22	0.02	0.05	0.001			linear; < 0.001
d 28	0.09	0.11	0.08	0.07	0.03	0.77			
d 42	0.17	0.16	0.19	0.11	0.02	0.16			
d 56	0.09	0.07	0.05	0.11	0.02	0.09			quadratic; 0.03
d 70	0.09	0.10	0.10	0.07	0.02	0.52			
d 84	0.19	0.17	0.19	0.21	0.02	0.64			
d 98	0.09	0.13	0.13	0.15	0.01	0.02			linear; 0.002
d 112	0.11	0.14	0.13	0.07	0.02	0.08			quadratic; 0.02
BW, d 112, kg	519.7	519.0	481.1	457.9	17.0	0.001			linear; < 0.001
$/kg of BW gain, d 0 to 69	$1.33	$1.39	$1.45	$1.94					
$/kg of BW gain, d 70 to 112	$1.98	$1.77	$1.78	$1.85					

[1]Effects of substituting ground juniper for ground alfalfa hay on steer BW. During Period 1, steers were fed a 70% concentrate diet from d 0 to 69 d that differed only by roughage source; juniper replaced 0, 33, 66 or 100% of the alfalfa (0JUN, 33JUN, 66JUN, or 100JUN, respectively). During Period 2 (d 70 to 112), steers were transitioned onto a common alfalfa-based 90% concentrate diet.

[2]T = treatment effect; T × D = treatment × day interaction.

[3]DMI = average daily DMI; $/kg of BW gain = cost per kg of diet/kg of BW gain.

[4]SEM = greatest standard error of the mean.

quantified (method 942.05; AOAC, 2006) and minerals analyzed by a Thermo iCAP 6300 Inductively Coupled Plasma Radial Spectrometer (Thermo Scientific, Inc., Waltham, MA) inductively coupled plasma radial spectrometer. True 48-hour *in vitro* DMD (tIVDMD) and neutral detergent fiber digestibility (NDFD; reported as % of NDF) were evaluated using cattle rumen fluid.

Condensed tannins (CT) in the mechanically dried (50°C) ground juniper and alfalfa subsamples were assayed for extractable, protein-bound, and fiber-bound fractions by methods described by Terrill et al. (1992). Standards were prepared as recommended by Wolfe et al. (2008) using CT extracts purified on a Sephadex LH-20 (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) and lyophilized to recover purified CT. Subsamples were also analyzed for protein precipitable phenolics (PPP; measure of readily bioactive tannins), amount of protein bound by CT/kg of material (PB), and protein binding capacity of CT (PB/PPP; CT potency) according to procedures of Naumann et al. (2014). Isocupressic acid (ICA), agathic acid (AGA), imbricatoloic acid (IMB), and dihydroagathic acid (DHAA) were analyzed at USDA-ARS Poisonous Plants Research Center, Logan, UT, according to methods described by Gardner and James (1999). Additional sub-samples (as-fed) were steam

distilled to determine total volatile oil yield according to methods described by Koedam and Looman (1980) and modified by Adams (1991).

Blood serum analysis. Blood samples were collected before feeding from each steer via jugular venipuncture using a nonheparinized vacutainer collection tube (serum separator tube, gel, and clot activator; Becton Dickenson, Franklin Lakes, NJ). Blood was allowed to clot before centrifuging (Eppendorf 5804, Hauppauge, NY) at 970 × g for 25 min at 4°C. Decanted serum was frozen at –20°C. Serum chemistry was analyzed by The Texas A&M Veterinary Diagnostic Laboratory, Amarillo, using an Olympus AU400E analyzer (Olympus America Inc., Center Valley, PA).

Statistical Analysis

Data were analyzed using PROC MIXED procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC) with a model that included treatment, day, and treatment × day interaction; day was the repeated measure and steer was the subject. Blood serum chemistry variables that did not have normally distributed residuals were analyzed using PROC GLIMMIX with the appropriate distribution; day was the random effect and steer was the subject. Differences in least squares means were evaluated using the DIFF option with a SIMULATE adjustment. When a treatment × day interaction was observed ($P < 0.10$), treatment effects were evaluated by day.

Appropriate covariance structures were compared to determine the best structure for each model. Data are reported as least squares means with greatest SE. Treatment effects were tested using linear, quadratic, and cubic orthogonal contrasts; only the highest order contrast that was significant ($P < 0.10$) was discussed.

RESULTS AND DISCUSSION

Steer Growth Performance

Treatment × day interactions ($P < 0.001$) were observed for steer DMI, ADG, and G:F. As percentage of juniper increased in the diet, daily DMI linearly decreased ($P < 0.01$) on d 14, 28, 42, 56, and 70. Steer DMI was similar ($P > 0.34$) among all steers after being transitioned onto the common alfalfa-based finishing diet (Period 2).

Reduced DMI was likely a result of extreme differences in particle (e.g., size, structural fiber, buoyancy, hydration rate) and digestive characteristics between the ground alfalfa and juniper. The juniper was ground through a smaller screen vs. the alfalfa, resulting in smaller particles. Thus, the ground juniper should have theoretically had greater passage rate than alfalfa. However, grinding can reduce physically effective NDF, thus chewing activity (Lammers et al., 1996). Furthermore, tIVDMD and NDFD of the ground alfalfa was 20 and 14 percentage units greater than the ground juniper, respectively, which was generally reflected in the diets. Reduced digestibility of the juniper-based diets, along with these diets containing greater concentrations of dense particles that settle in the ventral rumen, may have reduced passage rate, thus contributing to reduced DMI as discussed by others (King and Moore, 1957; Welch, 1986). This would be especially true for the 100JUN diet, which would not have contained enough buoyant particles to develop an effective rumen mat layer.

Reduced steer DMI may have also been a result of volatile oil, CT, or both, reducing diet palatability. Ground juniper contained 1.62% volatile oil and 2.67% CT vs. alfalfa that contained practically no volatile oil and only 0.22% CT (Table 1). Volatile oil (Utsumi et al., 2009; Estell et al., 2014) and CT (Terrill et al., 1989) have been reported to reduce DMI, either as a concentrated dose added to mixed feed or as a natural component of the plant. However, the volatile oil and CT concentrations of the juniper material, thus diets, were low and unlikely to be significant factors in the observed reduction in steer DMI.

As percentage of juniper increased in the diet, ADG linearly decreased ($P < 0.01$) on d 14 and 42, tended to linearly decrease ($P = 0.09$) on d 28, and tended to quadratically decrease ($P = 0.07$) on d 56. No differences ($P > 0.10$) in steer ADG was observed on d 70. Differences in steer DMI, in particular energy and protein intake, were likely the major factor affecting ADG.

After steers were transitioned onto the alfalfa-based finishing diet (Period 2), ADG linearly increased ($P < 0.001$) and tended to quadratically increase ($P = 0.07$) on d 98 and 112, respectively, for steers previously fed 0JUN, 33JUN, 66JUN, and 100JUN diets. Due to differences in steer ADG, negative linearly trends ($P < 0.01$) were observed for steer BW at each weigh date (d 14 to 112), resulting BW being less ($P < 0.05$) for steers fed 66JUN and 100JUN vs. steers fed 0JUN, at the end of each period (d 70 and 112; Figure 1). Compared to steers fed 0JUN, steers fed 33Jun, 66JUN, or 100JUN weighed 0.7, 38.6, 61.8 kg less at the end of the trial. If ADG of steers remained similar to that reported for d 112, then steers fed 66JUN and 100JUN would require an extra 31 and 81 days on feed, respectively, to reach the final BW (d 112) of steers fed 0JUN, which is not economically viable.

During Period 1, as percentage of juniper increased in the diet, G:F linearly decreased ($P < 0.001$) on d 14, quadratically decreased ($P = 0.03$) on d 56, and remained similar ($P > 0.10$) on d 28, 42, and 70. After steers were transitioned onto the alfalfa-based

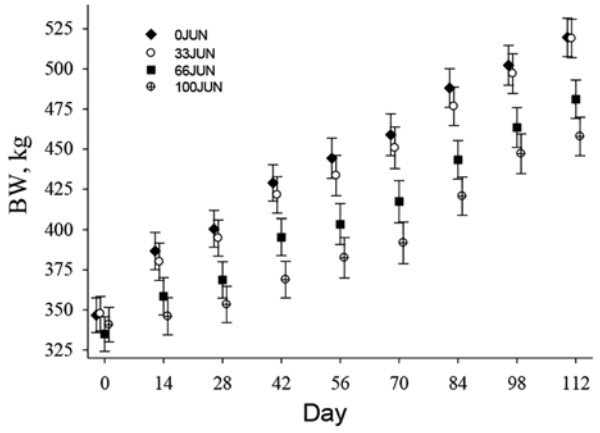

Figure 1. Effects of substituting ground juniper for ground alfalfa hay on steer BW. During Period 1, steers were fed a 70% concentrate diet from d 0 to 69 d that differed only by roughage source; juniper replaced 0, 33, 66 or 100% of the alfalfa (0JUN, 33JUN, 66JUN, or 100JUN, respectively). During Period 2 (d 70 to 112), all steers were transitioned onto a common alfalfa-based 90% concentrate finishing diet. A treatment × day interaction ($P < 0.001$) and linear trends ($P < 0.003$) were observed on each weight day from d 14 to 112.

finishing diet (Period 2), G:F was similar ($P > 0.10$) on d 84, but linearly and quadratically increase ($P < 0.03$) on d 98 and 112, respectively.

Steer Serum Chemistry Profiles

Steer serum chemistry profile data have been statistically analyzed, but data are not reported. In summary, substituting ground juniper for ground alfalfa hay in the feedlot growing diets (d 0 to 70) did not appear to negatively affect steer health as assessed by daily visual appraisal and blood serum chemistry.

Implications

Results indicated that replacing 66% or 100% of alfalfa hay in a steer growing diet with ground juniper would not be economical at the price of alfalfa observed during this trial. However, when assessing cost/kg of BW gain over the entire trial, the inclusion of 10% juniper (33JUN) should be considered economically viable. This is especially true during times when the price of roughage ingredients dramatically increase due to drought or seasonal availability.

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Comparison of growth, feed efficiency, and wool characteristics of commercial Rambouillet × South African Meat Merino crossbred rams to purebred Rambouillet rams

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ABSTRACT: Our hypothesis was crossbreeding purebred Rambouillet ewes with South African Meat Merino (SAMM) rams would increase growth and performance without reducing wool quality in composite offspring. Therefore, 88 weaned yearling ram lambs were used in a completely randomized design where rams with ≤ 25% SAMM genetic influence (LOW, n = 37) and rams with ≥ 50% SAMM (HIGH, n = 13) were compared to commercial 100% Rambouillet rams (CON, n = 38). Rams were individually fed and rams were weighed every 28 days for a total feeding period of 56 d. At the end of the testing period, wool samples were collected for analysis and comparison. Rams were shorn and grease fleece weights were measured. Initial and final BW did not differ between genetic groups (P = 0.25). Ram DMI did not differ (P = 0.39) over the course of the 56 d period. Nevertheless, 0 to 28 d ADG was greater (CON vs LOW and HIGH; P = 0.02) for SAMM crossed rams when compared to CON, irrespective of percentage of SAMM. However, 28 to 56 d ADG was greater for CON (CON vs LOW and HIGH; P = 0.05). Treatment had no effect on clean fleece weight (P = 0.21). Staple length did not differ across treatment (P = 0.56). Wool fiber diameter (side and britch) was coarser (CON vs LOW and HIGH; P < 0.001) for SAMM crossed rams irrespective of percentage SAMM. Average fiber diameter increased linearly as the percentage of SAMM influence increased. Wool comfort factor tended (P = 0.09) to be lower for the side sample and was lower (P = 0.01) for the britch sample of crossbred rams. In conclusion, crossing South African Meat Merino with fine wool Rambouillet did not increase growth performance and minimally impacted wool quality. Therefore, additional research is warranted to investigate progeny performance of lambs sired by South African Meat Merino × Rambouillet crossbred rams.

Key words: cross breeding, fine-wool breed, lamb performance, Rambouillet, South African Meat Merino

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INTRODUCTION

Optimization of lamb growth and production of a highly marketable fine wool fleece is essential to the success of the sheep industry. The Rambouillet breed is widely used in the southwestern United States because of its high quality fine-wool and hardiness (Leymaster, 2002). However, Rambouillet do not possess the growth characteristics of common meat breeds. Conversely, common meat breeds do not have the wool quality that many producers desire. Thus, crossbreeding may offer producers a method of improving growth and performance traits without hindering the potential of the fine-wool producing breeds. Redden et al. (2005) used Suffolk rams in a terminal crossbreeding program with fine-wool Western Whiteface ewes and reported an 8.5% greater weaning weight for crossbred lambs compared to purebred fine-wool lambs. However, terminal breeding systems can be labor intensive. Therefore, utilization of a dual purpose breed that maintains excellent growth and wool characteristics may be more suitable to range sheep production. The South African Meat Merino (SAMM) is a potential option. These sheep are known for their prolific growth and carcass characteristics and have successfully integrated into fine-wool operations in parts of South Africa and Australia with this very crite-

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rion in mind (Cloete and Durand, 2000). The addition of SAMM genetics has complimented operations in these parts of the world by contributing to greater lamb growth and lambing percentage (Brand and Franck, 1999; Ross et al., 2008) as well as improved carcass characteristics (Brand and Franck, 1999). However, the primary development of the SAMM breed has been established for meat production whereas wool production has remained a secondary trait of importance (Brand and Franck, 1999). Therefore, the literature is lacking in information regarding the impact of crossbreeding SAMM with fine-wool Rambouillet in Western U.S. sheep production systems. Our hypothesis was crossbreeding purebred Rambouillet ewes with SAMM rams would increase growth and performance without reducing wool quality in composite offspring. Our objectives were to compare growth and wool characteristics of rams that contained low or high percentages of SAMM to pure bred Rambouillet.

MATERIALS AND METHODS

All procedures were reviewed and approved by the New Mexico State University Institutional Animal Care and Use Committee. In three separate experiments during the spring of 2014, 2015, and 2016, a total of 88 weaned yearling rams (2014, n= 32; 2015, n= 31; 2016, n= 25) were transported from New Mexico State University's Corona Range and Livestock Research Center, Corona, NM to New Mexico State University, Las Cruces, NM. Rams were housed in individual feedlot pens and allowed ad libitum access to feed and water.

Animals

Eighty-eight weaned yearling ram lambs were housed in individual feedlot pens for 56 d in order to assess the effects of different levels of SAMM blood when crossed with Rambouillet. Rams with ≤ 25% SAMM genetic influence were considered low percentage (LOW, n = 37), while rams with ≥ 50% SAMM genetic influence were considered high percentage (HIGH, n = 13) and were compared to commercial 100% Rambouillet rams (CON, n = 38). Rams were fed to meet or exceed NRC requirements (NRC, 2007) for growing ram lambs. Initial and final BW were collected on two consecutive days. Daily feed intake was measured and rams were weighed every 28 d for a total feeding period of 56 d.

Wool Analysis

At study initiation 10 × 10 cm (50 g) side and britch samples were shorn off the fleece. At commencement of 56 d trial regrowth of wool was measured for staple length in 5 replicate measurements, averaged and adjusted to adjusted 365 d. Side samples were then shorn and analyzed. An Optical Fiber Diameter Analyzer 2000 (BSC Electronics Pty. Ltd., Attadale, Western Australia) was calibrated for opacity measurement using a single standard slide of known opacity provided by the instrument manufacturer. All of the side samples were cut with a guillotine to produce snippets (short pieces of fiber ≈ 2 mm in length) and then washed. These snippets were measured for average fiber diameter (AFD), SD of fiber diameter (FD-SD), comfort factor (CF), and curvature (CURV) according to the International Wool Textile Organization (IWTO, 2013). Grease fleece weights (GFW) were recorded at shearing. Estimated clean wool fibers present were analyzed (ASTM, 1990) on composited side and britch samples within breed type to calculate clean fleece weights.

Statistical Analysis

Growth performance, feed efficiency, and wool characteristics were all analyzed as a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Animal served as the experimental unit and the statistical model included the effect of treatment with year included in the RANDOM statement. Differences were considered significant when $P \leq 0.05$ and considered as tendencies when $P \leq 0.10$. Single degree of freedom orthogonal contrasts was used to compare effect of CON vs. SAMM HIGH and LOW. In addition, orthogonal polynomial contrasts were used to compare linear and quadratic response to percentage of SAMM (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

No differences ($P = 0.29$) were seen in final BW across treatment (Table 1). This disagrees with results found by Redden et al. (2005) where Suffolk × Western Whiteface lambs had heavier final body weights when compared to the Western Whiteface controls. Ram DMI intake did not differ ($P \geq 0.39$) between genotypes over the entire 56 d experiment. Daily BW gain from d 0 to 28 ADG was greater ($P = 0.02$) for SAMM than CON. This agrees with Brand and Franck (1999) who reported that SAMM influenced lambs had greater 45 and 100 d of age ADG. Conversely, in the second 28 d period, CON ADG was greater ($P = 0.05$) than SAMM, irrespective of LOW or HIGH percentages. Admittedly, DMI intake did not differ between treatments statistically, however, the slight numerical differences may have contributed to ADG differences observed. Specifically, SAMM rams consumed more DM

TABLE 1. Effects of crossbreeding Rambouillet ewes with South African Meat Merino (SAMM) rams on growth, feed efficiency, and wool characteristics of yearling rams

	Treatment[1]					Contrasts[2]		
Item	CON	LOW	HIGH	SE	*P*-Value	CON VS LOW and HIGH	L	Q
N	38	37	13	-	-	-	-	-
Initial BW, kg	47.6	48.7	48.9	4.7	0.49	0.25	0.43	0.76
Final BW, kg	67.7	69.7	69.4	2.8	0.29	0.15	0.40	0.49
DMI, kg								
d 0-28	1.64	1.68	1.72	0.14	0.69	0.39	0.48	0.95
d 28-56	2.46	2.48	2.40	0.15	0.90	0.87	0.71	0.67
d 0-56	2.05	2.08	2.06	0.14	0.90	0.75	0.93	0.74
ADG, kg								
d 0-28	0.323	0.366	0.403	0.053	0.07	0.02	0.05	0.94
d 28-56	0.379	0.378	0.334	0.039	0.14	0.05	0.06	0.67
d 0-56	0.360	0.363	0.376	0.057	0.58	0.50	0.92	0.42
G:F, kg								
d 0-28	0.209	0.224	0.234	0.038	0.47	0.22	0.34	0.90
d 28-56	0.165	0.154	0.143	0.032	0.18	0.06	0.12	0.97
d 0-56	0.180	0.183	0.176	0.033	0.96	0.87	0.99	0.83
Wool								
Grease fleece Wt., kg	3.95	3.90	3.69	0.37	0.36	0.19	0.16	0.57
Clean fleece Wt., kg	2.14	2.19	2.00	0.16	0.36	0.53	0.22	0.21
Average fiber diameter of side, µm	20.9	22.0	22.8	0.6	<0.01	<0.001	<0.001	0.72
Average fiber diameter of britch, µm	21.8	23.3	23.8	0.7	<0.01	<0.001	0.02	0.31
Side FD-SD, µm	3.8	3.8	4.1	0.3	0.10	0.07	0.03	0.27
Britch FD-SD, µm	4.2	4.3	4.6	0.4	0.31	0.18	0.13	0.47
Staple Length, cm (365 d Adjusted)[3]	13.4	13.8	14.5	1.3	0.56	0.29	0.32	0.86
Comfort Factor of side, %	97.5	97.8	94.5	0.9	<0.01	0.09	<0.01	0.01
Comfort Factor of britch, %	96.1	94.1	90.3	1.7	<0.01	<0.01	<0.01	0.50
Curvature of side, °·mm^{-1}	107.5	110.7	110.4	4.3	0.44	0.24	0.47	0.58
Curvature of britch, °·mm^{-1}	99.2	100.1	102.6	7.8	0.70	0.42	0.42	0.82

[1]Rams with ≤ 25% SAMM genetic influence were considered low percentage (LOW, n = 37), while rams with ≥ 50% SAMM genetic influence were considered high percentage (HIGH, n = 13) and were compared to commercial 100% Rambouillet rams (CON, n = 38).

[2]Contrasts: Control versus Low and High SAMM rams; L: Linear; Q: Quadratic.

[3]Staple length collected at the end of experiment and adjusted to 365 d.

during the first 28 d period, while CON rams consumed numerically more DM during the second 28 d period. Feed efficiency (G:F) tended ($P = 0.06$) to be greater between d 28 and 56 for CON. Whereas, G:F did not differ ($P \geq 0.18$) for d 0 to 28 and d 0 to 56. Growth performance of SAMM rams was greater in the first 28 d of the test period, which may reflect the high growth and early maturation of the SAMM breed. However, ADG of the Rambouillet was greater during the second 28 d which negated any growth advantage.

Grease fleece weight was not different ($P \geq 0.16$) between SAMM and CON. Clean fleece weight was not different between crossbred rams and CON rams ($P \geq 0.36$). Staple length, adjusted 365 d, did not differ ($P = 0.56$) between treatments. Rambouillet rams had finer side and britch AFD ($P < 0.01$ and $P = 0.02$ respectively) than LOW and HIGH rams. Rambouillet rams tested herein were similar to the fine wool Merino tested by Mullaney et al. (1969). Additionally, SAMM crosses were only 2 µm higher than Merino and were 6 µm less than Corriedale wool (Mullaney et al., 1969). Coarseness of both side and britch fibers increased linearly ($P < 0.01$ and $P < 0.01$) with increased influence of SAMM blood. Side FD-SD was more variable as percentage of SAMM increased ($P = 0.03$) however there was no variability observed between treatments in FD-SD for britch wool. Cloete and Durand (2000) reported that purebred SAMM lambs had lighter fleeces and were shorter in staple length and larger average fiber diameter compared to Merino. There were no observed differences of curvature of side or britch wool between differences. CON had a higher CF for side wool than either LOW or HIGH treatment (97.5%, 97.8%, and 94.5% respectively; $P < 0.01$). A quadratic reduction ($P = 0.01$) in CF was observed in side wool samples as percentage of SAMM

influence increased. Our hypothesis was that crossing high growth SAMM rams with fine wool Rambouillet would improve growth performance without reducing wool quality. Although wool characteristics did not change in regards to fleece weight and staple length, AFD did increase for SAMM rams. Nevertheless, rams from all genotypes produced wool congruent with high quality comfort factor standards described by Naylor (2010), which is desirable for worsted processing applications. Overall, differences in percent SAMM (LOW or HIGH) did not appear to increase or decrease post weaning growth performance and wool characteristics under genotypes represented in the current study. Additional research is warranted to determine additive gains in lamb survival, pre-weaning growth performance, and reproductive performance of SAMM influenced sheep.

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Postweaning feed efficiency decreased in progeny from high milk producing beef cows

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ABSTRACT: The objective of this study was to evaluate the effects of actual milk yield in mature beef cows on DMI, BW gain, ADG, feed conversion ratio (FCR), and residual feed intake (RFI) of the progeny during a ~75 d backgrounding period. Twenty-four hour milk production was measured at ~ d 58 of postpartum with a modified weigh-suckle-weigh technique using a milking machine. After milking, cows were retrospectively classified as 1 of 3 milk yield groups: Low (6.57 ± 1.21 kg), Moderate (9.02 ± 0.60 kg), or High (11.97 ± 1.46 kg). After weaning, calf BW was measured prior to entry into the GrowSafe Feeding System and again 10 d after to account for the acclimation period to the feeding system. The BW after the acclimation period was considered the initial entry BW. During the 75 d backgrounding period, steers and heifers were fed a corn silage-based growing ration (13.75% CP and 67.98% TDN, DM basis). After the acclimation period, BW were recorded at a mid-point (~35 d post-acclimation) and at the termination of the feeding trail (~75 d post-acclimation). Dry matter intake was calculated as the average of the dry matter consumed from the initiation of the study to the mid-point, mid-point to termination of the feeding study, and overall intake from initiation to termination. Calves from Moderate and High milking dams had greater ($P < 0.01$) BW from the initiation until the end of the backgrounding feeding phase; however, final BW were not different ($P = 0.36$) between Low and Moderate calves. Body weight gain was greater ($P = 0.05$) for Low and Moderate calves from the initial BW to midpoint BW compared to High calves. Overall DMI was lower ($P = 0.04$) in offspring from Low and Moderate cows compared to their High milking counterparts. With the decreased DMI, FCR was lower ($P = 0.03$) from initiation of feeding to the midpoint in calves from Low and Moderate milking dams. In addition, overall FCR was lower ($P = 0.04$) in calves from Low and Moderate milking dams compared to calves from High milking dams. However, Low calves had an increased ($P = 0.03$) efficiency from a negative RFI value compared to Moderate and High calves. Results from this study suggest that selecting for maternal traits of high milk production decreases feed efficiency during a 75 d post-weaning, backgrounding period.

Key words: beef cattle, feed efficiency, feed intake, milk production, postweaning growth
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INTRODUCTION

Maximizing profit through genetic selection of growth may have negative consequences for long-term success in the beef industry. These selection traits do impact profitability for cow-calf producers; however, calf BW at weaning, for instance, only accounts for 5% of profitability for the producer in a profit model (Miller et al., 2001). However, efficiency from birth to slaughter has not been consistent with increasing milk yield. Increasing milk yield has been reported to decrease (Montaño-Bermudez and Nielsen, 1990) and increase (Brown and Dinkel, 1982) efficiency. Selection for increased milk yield results in an increase in cow maintenance energy requirements (Neville and McCullough, 1969; Ferrell and Jenkins, 1985; Montaño-Bermudez and Nielsen, 1990). Thus, improvements in energy utilization and conversion efficiency by beef cow herd to optimize inputs and outputs would greatly increase efficiency of the beef industry. One area of research

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that is not quite defined is the optimum level of milk production in a given environment to optimize production efficiency from post-weaning. Therefore, the objective of this study was to evaluate the effects of actual milk yield in mature beefs cows on offspring dry matter intake, BW gain, and feed efficiency for a 75 d backgrounding period. The hypothesis was that offspring from high milk yield dams will decrease feed efficiency post-weaning.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of University of Tennessee, Knoxville approved all described animal handling and experimental procedures.

In a 2-yr study, 91 spring-born, weaned Angus steers and heifers from the Plateau Research and Education Center, Crossville, TN, USA were used to determine the influence of dam's milking potential on progeny performance and feed efficiency post-weaning. On approximately d 58 and 129 postpartum, cow milk yield was measured using a modified version of weigh-suckle-weigh method described by Mulliniks et al. (2011). Cows were milked using a portable milking machine (Porta-Milker, Coburn Company Inc., Whitewater, WI). On the day of the milking, cows were gathered from their pasture and calves were removed. Ten minutes before milking, cows were administered an intramuscular injection of oxytocin (20 IU; Vedo Inc., St. Joseph, MO) to facilitate milk letdown. Cows were milked until machine pressure could not extract any additional fluid, and milk collected was subsequently discarded. After first milking, cows were kept separate from calves and then milked a second time. Milk weights were recorded to calculate 24-h milk production. After milking, cows were retrospectively classified as 1 of 3 milk yield groups: Low (6.57 ± 1.21 kg), Mod (9.02 ± 0.60 kg), or High (11.97 ± 1.46 kg). A complete description of cow and calf management up to weaning is reported in Edwards et al. (2017).

At weaning each year, calves were vaccinated against bovine respiratory disease complex (bovine respiratory syncytial virus, infectious bovine rhinotracheitis, bovine viral diarrhea, and parainfluenza 3) with Bovi-Shield Gold 5 (Pfizer Animal Health, Exton, PA). Calves were administered a 7-way clostridial vaccine (Ultrabac-7, Pfizer Animal Health) and were vaccinated against Pasteurella (One-Shot; Pfizer Animal Health). Two weeks after weaning, steers and heifers were acclimated in the GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) for 10 d prior to measuring feed intake. Calf BW was measured prior to entry into the GrowSafe and again 10 d after to account for the acclimation period to the feeding system. The BW after the acclimation period was considered the initial entry BW. After the acclimation period, BW were recorded at a mid-point (~35 d post-acclimation) and at the termination of the feeding trail (~75 d post-acclimation). During both the acclimation and study period, steers and heifers were provided free-choice access to a corn silage-based ration (13.75% CP and 67.98% TDN, DM basis) and individual DMI was recorded daily. Random samples of the corn silage-based ration were collected at the beginning and end of the study, composited into a single sample, and analyzed in triplicate for nutrient content using wet chemistry procedures at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Dry matter intake was calculated as the average of the dry matter consumed from the initiation of the study to the mid-point, mid-point to termination of the feeding study, and overall intake from initiation to termination. Average daily gain and average DMI for each time point were utilized to calculate a feed conversion ratio (**FCR**; feed:gain) for individual animals.

Average daily live weight gain during the residual feed intake (**RFI**) measurement period for both heifers and steers was computed as the coefficient of the linear regression of BW (kg) on time (d) using the GLM procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Midpoint metabolic BW (**MBW**) was determined as $BW^{0.75}$ in the middle of the RFI measurement period, which was estimated from the intercept and slope of the regression line after fitting a linear regression line through all metabolic BW ($BW^{0.75}$) observations. Residual feed intake was calculated for each heifer and steer as the difference between actual DMI and expected DMI. Expected DMI was computed for each heifer and steer using a multiple regression model, regressing DMI on MBW and ADG.

Normality of the data distribution and equality of variances of measurements were evaluated using PROC UNIVARIATE and the Levene test and White's test, respectively. Data were analyzed as a complete randomized design, using a mixed procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Calf was used as the experimental unit with the Kenward-Roger degrees of freedom method. The model included fixed effects of milk treatment, sex of calf, year and their interactions. Separation of least squares means was performed by the PDIFF option of SAS when a significant ($P \leq 0.05$) effect of milk production classification was detected. Sex of calf did not interact with milk yield treatment for any measurement and was not discussed in this manuscript.

RESULTS

Level of dam's milk production influenced ($P < 0.01$; Table 1) initial BW at the beginning of the 75 d backgrounding phase. Steers and heifers from Moderate and High milking cows had similar initial BW with progeny from Low milking cows having the lightest BW. Moderate and High calves maintained greater ($P \leq 0.03$) BW until the end of the backgrounding phase in the GrowSafe feeding system.

Even though calves from Low milking cows had the lightest BW during the study, calves from Low milking cows had similar BW gain to calves from Moderate cows and greater ($P = 0.05$; Table 1) BW gain from the initial BW to mid-BW. However, BW gain was not different ($P \geq 0.45$) among offspring for the remainder of the feeding trial.

Dry matter intake from the initial to midpoint tended to be lower ($P = 0.08$; Table 1) in calves from Low and Moderate cows than calves from High milking cows. From midpoint until the termination of the 75 d feeding trail, DMI was not different ($P = 0.62$) among treatments. However, overall DMI was greater ($P = 0.04$) in calves from High milking dams with no differences between calves from Low and Moderate milking cows.

With the lower DMI, FCR was lower ($P = 0.03$; Table 1) from initiation of feeding to the midpoint in calves from Low and Moderate milking dams. From midpoint to termination of the 75 d feeding period, FCR was not different ($P = 0.44$) among calves from differing levels of milk producing dams. However, overall FCR during the 75 d feeding trial was lower ($P = 0.04$) in calves from Low and Moderate milking dams compared to calves from High milking dams. After the 75 d feeding trail, calves from Low milking dams resulted in lower ($P = 0.03$) RFI values than calves from Moderate or High milking dams.

TABLE 1. Effect of dam's milk production level on progeny's body weight, body weight gain, average daily gain, dry matter intake, and feed efficiency post-weaning

	Milk Production[1]				
	Low	Moderate	High	SEM	*P*-value
BW, kg					
Initial[2] BW	297[a]	317[b]	318[b]	8	< 0.01
Mid[3] BW	338[a]	361[b]	352[ab]	10	0.02
Final[4] BW	378[a]	400[b]	392[ab]	11	0.03
BW gain, kg					
Initial to Mid	41[a]	44[a]	34[b]	3	0.05
Mid to Final	40	36	34	5	0.90
Initial to Final	81	80	68	4	0.45
Average Daily Gain, kg/d					
Initial to Mid	1.23[a]	1.27[a]	1.13[b]	0.08	0.06
Mid to Final	0.74	0.77	0.74	0.08	0.92
Initial to Final	0.95	0.99	0.92	0.07	0.49
Dry Matter Intake, kg/d					
Initial to Mid	10.11[a]	10.39[a]	11.72[b]	0.40	0.08
Mid to Final	12.24	12.35	12.60	0.45	0.62
Initial to Final	11.02[a]	11.40[a]	12.41[b]	0.39	0.04
Feed Conversion Ratio[5]					
Initial to Mid	8.22[a]	8.19[a]	10.42[b]	1.22	0.03
Mid to Final	16.54	16.02	17.11	1.45	0.44
Initial to Final	11.60[a]	11.53[a]	13.52[b]	1.30	0.04
RFI[6]	-1.06[a]	0.33[b]	0.69[b]	0.46	0.03

[a,b]Means with differing superscripts differ among milk production groups ($P < 0.05$).

[1]Milk production groups: Low (6.57 ± 1.21 kg), Moderate (9.02 ± 0.60 kg), and High (11.97 ± 1.46 kg).

[2]Initial BW occurred after 10 d acclimation period in the GrowSafe feeding system.

[3]Mid BW occurred ~ 35 d after initial BW.

[4]Final BW occurred ~ 75 d after initial BW.

[5]Expressed as feed:gain.

[6]Residual feed intake.

DISCUSSION

The continual increase in selection for milk production has resulted in dairy and beef cows that are under greater nutritional stress in critical physiological periods, such as early lactation, that will ultimately reduce reproductive traits (Dillon et al., 2006; Edwards et al., 2017). Due to the impact of milk yield on calf weaning weight, cow-calf producers tend to emphasize a selection for increased genetic potential for milk in their cow herds. However, such emphasis may not increase overall efficiency of the entire beef cow industry.

Milk yield did influence post-weaning BW in the current study. Moderate and High milk yields had greater BW at the initiation of the backgrounding phase in the GrowSafe. However, after ~35 d in the study, progeny from Low milking beef cows did have similar BW than progeny from High milk yield cows for the rest of the feeding trial. In a simulated model, Bourdon and Brinks (1987) found that increasing beef cow milk production was favorable when feedlot feed costs were high but not when cow herd feed costs were high. However, this would be dependent on a linear positive relationship between milk yield and feedlot entry weight. Although, BW was significant in this study, increased milk yield did not linearly increase BW of the offspring. Final BW was not different between steers and heifers from low and high milk yielding dams.

Post-weaning gain did not respond linearly to increase in milk production. Low and moderate calves had greater BW gain and ADG during the first ~30 d of the feeding trial; however, this increase in ADG did not continue for the entire feeding trial. From the midpoint until the termination of the trial, BW gain and ADG were not different by milk yield of dams. In

dairy calves, an increase in pre-weaning milk intake decreased post-weaning ADG and FCR by more than 50% (Muya and Nherera, 2014). Wang et al. (2009a,b) illustrated that greater levels of milk yield was associated with decreased post-weaning backgrounding ADG. However, these authors do suggest that this may be dependent on both calf sire breed and post-weaning management (i.e., drylot vs wheat pasture).

Dry matter intake from the initiation of the study until midpoint did tend to decrease with offspring from Moderate and Low milk production cows. In contrast, Miller et al. (1999) reported that milk yield did not influence feedlot intake in steers. In contrast, due to a lack of intake difference, Miller et al. (1999) suggested that offspring from high milk producing cows were more energetically efficient than lower milk yielding cows. In contrast, FCR in the present study was increased for Low and Moderate offspring from the initiation to midpoint and overall FCR compared to High milk yield offspring. According to Montaño-Bermudez and Nielsen (1990), when production efficiency was estimated as weight of calf weaned per unit of energy intake, lower-milking cows were more efficient producers to weaning; the calves retained this efficiency advantage through the feedlot. This efficiency advantage to weaning appears to remain throughout the lifetime production of the lower-milking cows (Davis et al., 1983a,b). In agreement, Oijen et al. (1993) showed that offspring from moderate and high milk yielding beef cows were approximately 3 to 4% lower at weaning and 7% lower at slaughter than the offspring from low milk yield cows for biological efficiency.

Residual feed intake across milk production treatments indicated steers and heifers from low milk producing dams have an increased feed efficiency during backgrounding. Offspring from Moderate and High milking dams both showed to have positive RFI values. Ferrell and Jenkins (1984) have reported that efficiency of energy use is reduced in higher-milking beef cows. This research indicated that decrease in efficiency is a result of increased size of visceral organ mass (i.e., heart, liver, kidney, rumen, and small and large intestines). Increased feed intake and gut capacity are related to increased visceral organ mass relative to live body weight (Wang et al., 2009a,b). Digestive tract tissue and the liver use approximately 40 to 50% of total energy expenditure in the beef cow, even though these tissues make up less than 10% of the animal's body mass (Ferrell, 1988).

The increase in feed efficiency in Low calves may be due to rumen development differences. Development of the rumen is an important physiological process for young ruminants. Insufficient rumen development will negatively affect nutrient digestion and absorption (Baldwin et al., 2004). In calves from low milk producing cows, Ansotegui et al. (1991) reported no differences in ADG of calves from low milk producing cows versus high milk producing cows after d 60, due to forage intake differences. Buskirk et al. (1995) indicated that milk consumption was inversely related to forage intake. Likewise, Tedeschi and Fox (2009) indicated that there is an inverse relationship between milk consumption and forage intake, but milk was prioritized over forage intake if both are readily available. Wyatt et al. (1976) reported that high milk consumption reduced forage intake as much as 50%. Therefore, if milk production is limited, then calves will increase their forage intake, which may have an influence on rumen development. Forage consumption promotes muscular development of the rumen (Hamada et al., 1976) and stimulates rumination and flow of saliva into the rumen (Hodgson, 1971). In young dairy calves, the addition of forage to a grain starter diet improved feed efficiency (Coverdale et al., 2004); however no long-term influences were studied. The possible increase in forage intake pre-weaning may have provided the opportunity to adapt to mixed rations in the GrowSafe feeding system, which may explain the differences the first ~30 d of the feeding trial.

IMPLICATIONS

This study does reveal that selecting for high milk yield for increased calf growth results in a decreased post-weaning feed efficiency. Coupling RFI and FCR data, offspring from low milking beef cows have an increased post-weaning feed efficiency. Combining the cow performance results of Edwards et al. (2017) and results in this study, discounting the selection for milk production increases cow herd efficiency through the backgrounding phase. In addition, cow-calf or backgrounding systems may need to consider cow herd milk production to optimize post-weaning calf production and efficiency. Therefore, balancing genetic potential for milk yield with post-weaning performance of the offspring is necessary to develop efficiency in all beef industry segments.

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Impact of pre- and postpartum nutrition on March-calving cow and progeny productivity

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ABSTRACT: Crossbred March-calving cows (yr 1, n = 72; yr 2, n = 65; yr 3, n = 64) were used in a 3-yr experiment to evaluate the effect of grazing corn residue prepartum and subirrigated meadow postpartum vs harvested meadow hay during both periods on cow pregnancy rates and calf feedlot performance. A 2×2 factorial arrangement of treatments was used. From Dec 1 to Feb 28 (prepartum), cows grazed corn residue or were fed ad libitum hay (7.7% CP and 56.8% TDN). From parturition to the completion of a 45 d breeding season (July 20), half of the cows were fed ad libitum hay and the remainder grazed subirrigated meadow. Cow BW and BCS were monitored throughout the year and calf performance was recorded through slaughter. No interactions were present between pre- and postpartum treatments. Cows fed hay prepartum (HPRE) gained more BW and BCS than cows grazing corn residue (CPRE) during the prepartum period ($P < 0.01$). Cows fed hay prepartum had greater precalving and prebreeding BW and BCS than CPRE cows ($P < 0.01$). A greater BW and BCS change postpartum (May 15 to Nov 1) was seen in CPRE vs HPRE cows ($P < 0.01$). Cows that grazed meadow postpartum (MPOST) had greater BW and BCS at prebreeding and weaning (Nov 1) than cows fed hay postpartum (HPOST, $P < 0.01$). On Dec 1, MPOST cows also had greater BW and BCS than HPOST cows ($P < 0.02$). Pregnancy rates were similar for pre- or postpartum treatments ($P > 0.50$). Cows on the CPRE treatment tended to have an earlier calving date ($P = 0.06$) and a greater percentage calving in the first 21 d ($P = 0.07$) than HPRE cows. There were no differences in calf BW, weaning rate, or ADG for prepartum treatments ($P > 0.15$). Calves born to MPOST cows had greater birth ($P = 0.05$), prebreeding ($P < 0.01$), and weaning BW ($P < 0.01$) than HPOST calves. Calves from MPOST also had greater ADG prebreeding ($P = 0.01$) and from birth to weaning ($P < 0.01$). Steers from HPRE cows had a greater marbling score than CPRE steers ($P = 0.02$). All other feedlot and carcass characteristics were similar and not affected by pre- or postpartum treatments.

Key words: feedlot performance, postpartum nutrition, prepartum nutrition, reproduction
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INTRODUCTION

Feed costs are one of the greatest inputs in beef production systems. High costs of grazed forage have necessitated the evaluation of alternative systems. Corn residue can be utilized in many areas as a more economical feed source. Larson et al. (2009) observed increased BW and BCS in cows grazing corn residue in the prepartum period and pregnancy rates similar to cows grazing winter range. Stalker et al. (2006) evaluated feeding hay or grazing subirrigated meadow postpartum. Greater gains in BW and BCS were seen in cows grazing meadow, however, no differences in pregnancy rate were observed. Cows only remained on postpartum treatments for 30 d prior to breeding. Possible improvements in reproductive performance may have been seen if cows remained on treatments through the breeding season.

The importance of pre- and postpartum nutrition on the rebreeding performance of multiparous beef cows has been recognized by previous researchers (Corah et al., 1975; Randel, 1990; Morrison et al., 1999; Stalker et al., 2006). Cow BCS at calving is a good indicator of the cow's capability to rebreed, however postpartum nutrition can also influence reproduction (Wiltbank et al.,

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1962; Stalker et al., 2006). Nutrition during the pre- and postpartum segments of beef production and their interactions can also impact calf performance.

Objectives of this study were to evaluate systems that reduced the use of high cost grazed forage in the pre- and postpartum period. The effects of feeding hay or grazing corn residue prepartum and feeding hay or grazing subirrigated meadow postpartum on cow reproduction and subsequent calf productivity in a March-calving herd were evaluated.

MATERIALS AND METHODS

Animal use and management were in accordance with the University of Nebraska Institutional Animal Care and Use Committee guidelines. March-calving multiparous, Husker Red (3/4 Red Angus, 1/4 Simmental) cows (yr 1, n = 72; yr 2, n = 65; yr 3, n = 64) were blocked by age and allotted to 1 of 2 prepartum (Dec 1 to Feb 28) treatments: ad libitum hay (7.7% CP and 56.8% TDN, **HPRE**) or corn residue (0.61 AUM/ha, **CPRE**). Prior to calving, cows were vaccinated against Clostridium perfringens C/Escherichia coli/Rotavirus/Coronavirus (ScourGuard 4KC, Zoetis, Florham Park, NJ). From Feb 28 (precalving) until parturition, cows were managed in a common group and fed grass hay in a drylot. Each of these groups was split postpartum and half received ad libitum hay (**HPOST**) or grazed subirrigated meadow (**MPOST**). Subirrigated meadow used in this study was similar to that described by Volesky et al. (2004). At the beginning of breeding, cows were vaccinated against rhinotracheitis (**IBR**) virus, bovine virus diarrhea (**BVD**) virus (Types 1 and 2), *Campylobacter fetus* and *Leptospira canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, and *L. pomona* (Vista 3 VL5 SQ, Merck, Kenilworth, NJ). Cows remained on postpartum treatments from parturition through a 45 d breeding season (July 20). After this cows were managed as one group grazing native upland range until calves were weaned Nov 1.

Weight and BCS (Wagner et al., 1988) of all cows were recorded at the beginning (Dec 1) and end (Feb 28, precalving) of the prepartum period, prebreeding (May 15), and weaning (Nov 1). Cows received an ivermectin pour-on for internal and external parasites (Promectin B, Vedco, St. Joseph, MO) at prebreeding and weaning. A veterinarian diagnosed pregnancy via rectal palpation at weaning.

Calves were weighed at birth, prebreeding, and weaning. Calves received a 7-way clostridial vaccine (Alpha 7, Boehringer/Ingelheim, Duluth, GA) at birth. Bull calves were castrated and received an IBR, BVD Types I and II, bovine parainfluenza virus-3 (**PI3**), bovine respiratory syncytial virus (**BRSV**), Mannheimia haemolytica and Pasteurella multocida (Vista Once SQ, Merck, Kenilworth, NJ) and 7-way clostridial vaccine (Vision 7, Merck, Kenilworth, NJ) at branding (May 1). At weaning, steers (yr 1, n = 35; yr 2, n = 33; yr 3, n = 32) received 2 doses of Vista Once SQ 14 d apart and a 7-way clostridial with somnus (Vision 7 Somnus, Merck, Kenilworth, NJ). Steer calves remained in drylot on ad libitum hay for 2 weeks post weaning before being shipped 167 km to a feedlot at the West Central Research and Extension Center, North Platte, NE. Steers received a Synovex Choice (100 mg trenbolone acetate (**TBA**) and 14 mg estradiol benzoate (**EB**)) at the beginning of the feeding period. Steers were reimplanted with Synovex Plus (200 mg TBA and 24 mg EB) 105 d later (110 d prior to harvest). Steers were weighed at feedlot entry and reimplant. Steers were on a finishing diet similar to Larson et al. (2009). Hot carcass weight was determined at harvest; carcass characteristics were evaluated 24 h following harvest. Final BW was calculated from HCW, based on an average dressing percent of 63%.

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC) as a 2×2 factorial arrangement of treatments in a completely randomized design.

RESULTS AND DISCUSSION

Cow Variables

There were no interactions between pre- and postpartum treatments for cow variables (Table 1). Cows on HPRE gained more BW (39 ± 8 kg) and BCS (0.52 ± 0.13) than cows on CPRE during the prepartum period ($P < 0.01$). Cows on HPRE weighed more and had greater BCS precalving than CPRE cows ($P < 0.01$; 556 vs 512 ± 8 kg and 5.78 vs 5.20 ± 0.11 BCS for HPRE and CPRE, respectively). Crude protein and TDN values of hay (7.7% CP and 56.8% TDN) were greater than previously reported values for corn residue (Larson et al., 2009; 5.2% CP and 52.7% TDN), likely accounting for much of this difference. Cows on HPRE maintained a greater BW and BCS prebreeding ($P < 0.02$; 502 vs 487 ± 7 kg and 5.40 vs 5.09 ± 0.11 BCS for HPRE and CPRE, respectively). However, CPRE cows had greater BW gain and BCS postpartum (May 15 to Nov 1) than HPRE cows ($P < 0.01$; 21 vs 16 ± 4 kg for SPRE vs HPRE, respectively) likely due to a compensatory gain effect. These data are in agreement with Stalker et al. (2006) who reported cows receiving a protein supplement prepartum had greater BW and BCS at precalving and prebreeding and similarly, nonsupplemented cows had greater BW and BCS gain in the postpartum period. Freetly et al. (2000) reported compensatory gain of cows with re-

TABLE 1. Body weight, BCS, and reproductive performance of cows fed hay or allowed to graze corn residue prepartum or fed hay or allowed to graze subirrigated meadow postpartum

	Hay[1]		Residue[2]			*P*-value[3]		
Item	Hay[4]	Meadow[5]	Hay	Meadow	SEM	Pre	Post	Pre × Post
Cow BW, kg								
Dec. 1	483	506	484	494	7	0.46	0.02	0.35
Precalving	543	568	509	515	8	<0.01	0.10	0.25
Prebreed	484	519	479	494	7	0.02	<0.01	0.15
Wean	504	532	507	526	6	0.82	<0.01	0.48
BW change, kg								
Prepartum	60	62	25	20	8	<0.01	0.86	0.65
Postpartum	19	13	29	33	4	<0.01	0.79	0.19
Cow BCS								
Dec 1	5.07	5.41	5.02	5.34	0.08	0.47	<0.01	0.90
Precalving	5.73	5.83	5.18	5.22	0.11	<0.01	0.54	0.81
Prebreed	5.22	5.58	4.89	5.29	0.11	<0.01	<0.01	0.74
Wean	5.37	5.69	5.31	5.68	0.11	0.63	<0.01	0.74
BCS change								
Prepartum	0.66	0.41	0.16	-0.12	0.13	<0.01	0.08	0.90
Postpartum	0.14	0.11	0.43	0.39	0.08	0.02	0.75	0.95
Pregnancy rate, %	96	94	98	96	3	0.56	0.50	0.88
Calving date, Julian d	82	81	78	80	1.5	0.06	0.62	0.37
Calved 1st 21 d, %	66	71	82	77	6	0.07	0.99	0.40

[1]Cows fed ad libitum hay from December 1 to February 28 (prepartum).

[2]Cows grazed corn residue prepartum.

[3]Pre = prepartum treatment main effect; Post = postpartum treatment main effect; Pre × Post = prepartum × postpartum treatment interaction.

[4]Cows fed ad libitum hay from parturition to the completion of a 45 d breeding season (July 20, postpartum).

[5]Cows grazed subirrigated meadow postpartum.

stricted intake from the beginning of the second trimester until d 28 of lactation. Restricted cows had greater gains from 28 d to 205 d than nonrestricted cows. At 205 d postpartum, restricted cows had similar BW as nonrestricted cows.

Cows on MPOST had a greater BW and BCS at prebreeding and weaning than cows on HPOST ($P < 0.01$; 507 vs 482 ± 7 kg and 5.44 vs 5.06 ± 0.11 BCS at prebreeding; 529 vs 506 ± 6 kg and 5.69 vs 5.34 ± 0.11 BCS at weaning for MPOST vs HPOST, respectively). Esophageal fistulated cattle were used to quantify the nutritional quality of subirrigated meadow adjacent to the meadow pasture used in this study. In June, quality was 16.3% CP and 67.7% TDN. July values were 13.5% CP and 62.9% TDN. These values are much greater than the hay at 7.7% CP and 56.8% TDN, accounting for the differences seen in BCS and BW for MPOST cows. This difference carried through Dec 1 as MPOST cows had greater BW and BCS than HPOST cows ($P < 0.02$; 500 vs 484 ± 7 kg and 5.38 vs 5.05 ± 0.08 MPOST vs HPOST, respectively).

Despite differences in BW and BCS, pregnancy rates for pre- or postpartum treatments were similar ($P \geq 0.50$, Table 1). Cows on CPRE tended to have an earlier calving date ($P = 0.06$; d 79 vs 82 ± 1.5 CPRE vs HPRE, respectively) and a greater percentage calving in the first 21 d ($P = 0.07$; 80 vs 69 ± 6 % CPRE vs HPRE, respectively) possibly due to a flushing effect resulting in greater gain postpartum which may have had a stimulatory effect on estrus activity (Wiltbank et al., 1962).

Calf Variables

There were no interactions between pre- and postpartum treatments for calf variables. Calf birth, prebreeding and weaning BW; weaning rate; and ADG were similar for prepartum treatments ($P \geq 0.16$, Table 2). Calves born to MPOST cows had greater birth ($P = 0.05$), breeding, ($P < 0.01$) and weaning ($P < 0.01$) BW than HPOST calves and greater ADG ($P < 0.01$) prebreeding ($P = 0.01$) and from birth to weaning ($P < 0.01$). Stalker et al. (2006) observed a greater weaning BW and ADG to weaning for calves born to cows that grazed subirrigated meadow for 30 d postpartum compared with those fed hay during the same period.

Feedlot Performance

No interactions were present between pre- and postpartum treatments for feedlot performance. Even though differences ($P < 0.01$) were observed in weaning BW for MPOST (253 ± 4 kg) vs HPOST (239 ±

TABLE 2. Preweaning growth performance of calves born to cows fed hay or allowed to graze corn residue prepartum or fed hay or allowed to graze subirrigated meadow postpartum

	Hay[1]		Residue[2]			*P*-value[3]		
Item	Hay[4]	Meadow[5]	Hay	Meadow	SEM	Pre	Post	Pre × Post
Calf BW, kg								
Birth	35	37	34	36	1	0.23	0.05	0.75
Prebreed	179	204	178	198	3	0.60	<0.01	0.66
Wean	241	256	237	250	4	0.16	<0.01	0.81
Calf ADG, kg/d								
Birth to Prebreed	1.12	1.36	1.15	1.32	0.05	0.82	0.01	0.53
Prebreed to Wean	0.87	0.88	0.85	0.87	0.01	0.16	0.10	0.93
Birth to Wean	0.92	0.98	0.91	0.95	0.01	0.22	<0.01	0.74
Wean Rate, %	91	98	94	98	3	0.59	0.12	0.59

[1]Calves from cows fed ad libitum hay from December 1 to February 28 (prepartum).

[2]Calves from cows grazed corn residue prepartum.

[3]Pre = prepartum treatment main effect; Post = postpartum treatment main effect; Pre × Post = prepartum × postpartum treatment interaction.

[4]Calves from cows fed ad libitum hay from parturition to the completion of a 45 d breeding season (July 20, postpartum).

[5]Calves from cows grazed subirrigated meadow postpartum.

TABLE 3. Feedlot performance and carcass characteristics of steer calves born to cows fed hay or allowed to graze corn residue prepartum or fed hay or allowed to graze subirrigated meadow postpartum

	Hay[1]		Residue[2]			*P*-value[3]		
Item	Hay[4]	Meadow[5]	Hay	Meadow	SEM	Pre	Post	Pre × Post
Steer BW, kg								
Feedlot entry	251	256	246	257	5	0.68	0.11	0.61
Re-implant	435	433	427	442	10	0.95	0.55	0.46
Final	607	588	602	595	13	0.92	0.31	0.67
Steer ADG, kg/d								
Entry to re-implant	1.74	1.70	1.73	1.76	0.07	0.70	0.96	0.58
Re-implant to final	1.59	1.51	1.57	1.53	0.05	0.92	0.25	0.74
Overall	1.68	1.58	1.67	1.61	0.04	0.88	0.06	0.64
HCW, kg	382	371	380	375	8	0.92	0.31	0.67
12th rib fat, cm	1.45	1.42	1.47	1.32	0.15	0.83	0.69	0.73
Marbling[6]	504	470	424	450	20	0.02	0.86	0.16
LM, cm^2	86	86	87	87	2	0.74	1.0	0.94
Yield Grade	3.30	3.21	3.26	3.03	0.24	0.68	0.55	0.80
USDA Choice, %	94	77	64	73	20	0.47	0.86	0.56

[1]Steers from cows fed ad libitum hay from December 1 to February 28 (prepartum).

[2]Steers from cows grazed corn residue prepartum.

[3]Pre = prepartum treatment main effect; Post = postpartum treatment main effect; Pre × Post = prepartum × postpartum treatment interaction.

[4]Steers from cows fed ad libitum hay from parturition to the completion of a 45 d breeding season (July 20, postpartum).

[5]Steers from cows grazed subirrigated meadow postpartum.

[6]Where 400 = small^0.

4 kg), feedlot entry weights were similar (P = 0.11). This is in contrast to Stalker et al. (2006), who reported greater weaning BW and feedlot entry BW for steers on meadow treatment postpartum. Steers from HPOST tended (P = 0.06, Table 3) to have greater ADG throughout the feedlot period, compensating for lower gains in the postpartum period and resulting in similar final BW and HCW (P = 0.31, Table 3). Steers from HPRE cows had a greater marbling score than CPRE steers (P = 0.02; 487 vs 437 ± 20 for HPRE vs CPRE, respectively). Larson et al. (2009) observed greater marbling scores in steers from cows receiving protein supplement prepartum than those from unsupplemented cows. Supplemented cows would have been on a higher plane of nutrition as would the HPRE cows in the current study. This could explain the greater marbling scores observed in the present study. No

differences in any other feedlot variables were observed between pre- and postpartum treatments.

Implications

Results of this study demonstrate combinations of feeding hay or grazing corn residue prepartum, and feeding hay or grazing subirrigated meadow postpartum, impact BW and BCS during the pre- and postpartum period however, result in similar pregnancy rates. Although differences were not seen in cow pregnancy rates, a benefit in growth pre-weaning was seen for calves from the postpartum meadow treatment. An improvement in marbling score was observed for steers born to cows fed hay prepartum, indicating a higher level of nutrition prepartum may improve quality grade.

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Comparison of C and D_{65} illuminants of Minolta colorimeter for assessing pork color

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ABSTRACT: The objective of this study was to investigate the difference between two illuminants (C and D_{65}) of the Minolta colorimeter when assessing pork color. Pork chop samples (n = 2488) were assessed on the cross-sectional surface for instrumental color (CIE L*, a*, b*) using a Minolta colorimeter and both C and D_{65} illuminants. Instrumental color values were highly correlated between illuminants. The results showed there was a significant ($P < 0.0001$) yet numerically small difference between illuminants C and D_{65} regarding instrumental color values for measuring pork color. However, color values were highly correlated between C and D_{65} illuminants ($r > 0.96$), indicating similarities between the 2 illuminants. Therefore, it may be more important to establish independent research group relationships of instrument color values to other traits of interest than using a specific illuminant.

Key words: correlation, illumination, pork color
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INTRODUCTION

Color is an important part of the pork industry from its association with pork quality attributes (Sun et al., 2016) to consumer purchasing decisions (Mancini and Hunt, 2005). Currently, there are 2 primary methods to evaluate pork color: subjective and instrumental. The subjective method requires trained evaluators to assess pork color using the National Pork Board standards (NPB, 2011). The instrumental method uses colorimeters such as the Minolta (Chiyoda-ku, Tokyo, Japan) or Hunter (Reston, VA, USA) to assess pork color. According to the AMSA meat color measurement guideline, colorimeters measure tristimulus values (L*, a*, b*) under a certain illuminant (AMSA, 2012). The illuminant used can have an impact on the color measurements, with illuminant A having the highest correlations with visual color scores (Tapp et al., 2011). However, with the Minolta colorimeter, illuminants C and D_{65} are the most frequently reported in meat research articles (Tapp et al., 2011). Previous research has compared the difference in color values that come from varying instruments (Van Oeckel et al., 1999; Brewer et al., 2001; Mancini and Hunt, 2005) but little research has been conducted on the effect of illuminant on color values (Brewer et al., 2001; García-Esteban et al., 2003. In this study, 2 illuminant sources (C and D_{65}) were compared for assessment of pork color.

MATERIALS AND METHODS

Boneless center-cut pork loin 4-in sections (n = 2488) were collected. Two 1-in chops were cut from the center for data collection. For this study, one chop was allowed to bloom for a minimum of 10 min after being cut. Instrumental color values (CIE L*, a*, b*) were collected using a Minolta Colorimeter (CR-410, 50 mm diameter orifice, 2° observer, Minolta Company, Ramsey, NJ) on the cross-sectional surface. Both illuminants C and D_{65} were used on each pork chop sample to measure the color values.

Pearson correlation coefficients and simple statistics of L*, a*, and b* values for both the C and D_{65} illuminants were obtained using PROC CORR in SAS (v. 9.4, SAS Institute Inc., Cary, NC). Differences between illuminants for L*, a*, and b* were tested using the mixed procedure in

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TABLE 1. Simple statistics of instrument color values (L*, a*, b*) for both C and D_{65} illuminants

Trait	Mean	SD	Minimum	Maximum
C illuminant				
L*	53.68	3.22	43.28	67.20
a*	16.38	1.16	11.88	21.19
b*	6.98	1.19	3.58	12.42
D_{65} illuminant				
L*	53.76	3.24	42.75	67.36
a*	16.72	1.16	12.15	21.44
b*	6.66	1.18	3.37	12.17

TABLE 2. Least squares means of color values (L*, a*, b*) for C and D_{65} illuminants

Trait	C	D_{65}	SED	*P*-value
L*	53.69	53.77	0.013	<0.0001
a*	16.44	16.78	0.006	<0.0001
b*	7.04	6.72	0.007	<0.0001

SAS. Fixed effects included illuminant and evaluation date. The interaction between illuminant and evaluation date was tested and removed from the model since $P > 0.10$. A random effect of sample identification number was fitted in the model. The LS MEANS statement was used to test for differences between fixed effects. Values were considered different at $P < 0.05$.

RESULTS AND DISCUSSION

Simple statistics (mean, standard deviation, ranges) of the 6 instrument pork color values are reported in Table 1. Values of L*, a*, and b* were similar between the two illuminants. Because of the large number of samples and the minimum amount of variation, significant but small differences were found between C and D_{65} illuminants for L*, a*, and b*.

Pearson correlations were significant at the $P < 0.0001$ level. For L*, the correlation between C and D_{65} illuminants was 0.98. For a*, the correlation between C and D_{65} illuminants was 0.97. For b*, the correlation between C and D_{65} illuminants was 0.96. Scatter plots for L*, a*, and b* are shown in Figures 1 through 3. These results show a high correlation between illuminants for all color values (L*, a*, b*).

In a study by García-Esteban et al. (2003), the number of samples with a coefficient of variation less than 20 % was similar between illuminants A, C, and D_{65}. However, García-Esteban et al (2003) did not directly compare the different illuminants. In a study by Brewer et al. (2001), there was no difference in L* between C and D_{65} illuminants while a* was greater and b* was less for the D_{65} illuminant when compared to the C illuminant. While the use of the D_{65} illuminant resulted in a higher L* which was different from Brewer et al. (2001), a* was higher and b* lower in this study for D_{65} illuminant compared to C illuminant which is similar to Brewer et al. (2001). However, in this study, high, positive correlations for L*, a*, and b* values between C and D_{65} illuminants were found, suggesting a strong similarity between the two illuminants.

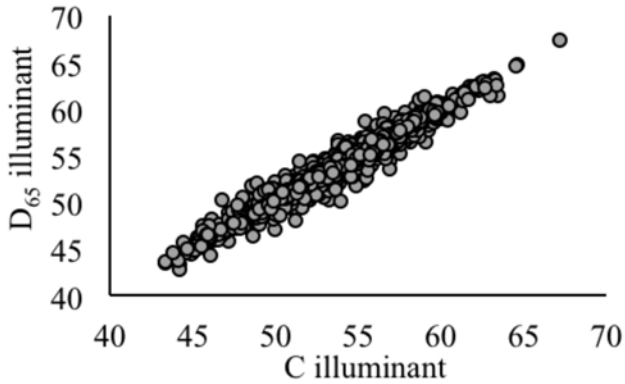

Figure 1. C versus D_{65} illuminants for L* (0 = black, 100 = white).

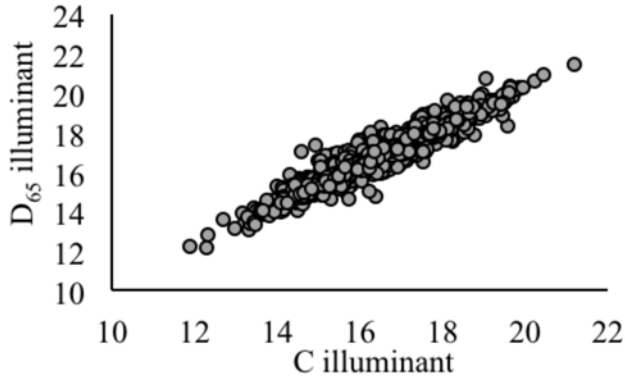

Figure 2. C versus D_{65} illuminant for a* (negative = green, positive = red).

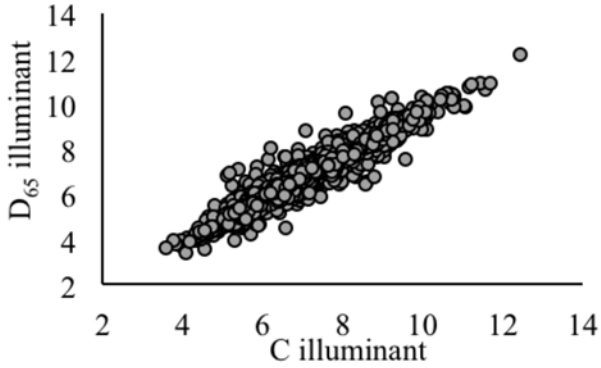

Figure 3. C versus D_{65} illuminate for b* (negative = blue, positive = yellow).

According to Tapp et al (2011), D_{65} was the most commonly used illuminant in pork studies which reported the illuminant used. However, the majority of studies (48.9 %) did not report the illuminant used (Tapp et al., 2011). However, other instrument devices like the Hunter, different aperture sizes, and different observer angles were not considered in this study. The implications of this study combined with results from other studies that illuminant used may not be as important as establishing the relationship between the instrument and illuminant used and other traits of interest for each research group. Results from one study may not be applicable to another study if different instrumentation is used.

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Effects of frequency of protein supplementation on performance by beef calves grazing dormant native range

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ABSTRACT: We evaluated growth performance of beef calves weaned onto dormant, native, tallgrass range supplemented with an average of 0.31 kg CP daily throughout the winter months. Calves (n = 233; 162 ± 21 d of age) were assigned randomly to 1 of 2 protein-supplementation treatments: daily supplementation (7X) or 3× week (3X) for 157 d. Calves assigned to both treatments grazed a single native tallgrass pasture. Calves were gathered at 0700 daily and sorted into two pens by treatment. Sunflower meal (SFM; 93% DM, 32.1% CP) pellets were fed to both treatments at a rate of 7 kg DM/calf weekly and were hand-delivered into concrete bunks (46 cm linear bunk space/calf). Calves assigned to 7X were sorted and group-fed 1 kg SFM/calf daily. Calves assigned to 3X were penned and sorted daily, but group-fed 2.3 kg SFM/calf on Monday, Wednesday, and Friday only. Calves were individually weighed following a 24-h period without access to feed at 28-d intervals throughout the study. Calf BW was not different ($P \geq 0.31$) between treatments at any time during the study, nor was calf BW change ($P = 0.49$) over the duration of the study. Calf ADG was not different ($P \geq 0.22$) between treatments from d 0 to 28, d 29 to 56, d 57 to 91, or d 118 to 157; moreover, ADG from d 0 to 157 was not different ($P = 0.48$) between treatments. In conclusion, daily protein supplementation did not improve growth performance relative to thrice-weekly protein supplementation, when weekly CP delivery was held constant between treatments. Feed delivery to 7X calves cost an estimated $1.75 / calf weekly, whereas feed delivery to 3X calves cost $0.75 /calf weekly.

Key words: pasture, protein, supplementation frequency

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INTRODUCTION

Stocker calves that graze forages before entering a feedlot account for more than 75% of the beef calves raised in the United States each year (Peel, 2000). A large percentage of those will be calves born in the spring and weaned in the fall. Modest growth rates are expected when the quality of fall and winter forages is poor (Krysl et al., 1989). Growing calves in confinement systems during fall winter typically allows for greater ADG than grazing low-quality forages (Bailey et al., 2012); however, modest overall costs associated with grazing perennial, dormant forages may be competitive during times when feed prices are relatively high.

Providing supplemental protein to beef cows grazing dormant, warm-season, native forages (i.e., ≤ 6% CP) increased BCS and BW (Mathis et al., 1999) and improved DMD and forage DMI (Köster et al., 1996). Furthermore, Beef cows grazing low-quality forages and supplemented with protein either daily, every third d, or every sixth d had similar BW and BCS (Huston et al., 1999; Schauer et al., 2005; Bennett et al., 2013).

Varying the frequency of supplement delivery can reduce labor costs and equipment depreciation without negatively affecting animal performance; however, this practice has met with variable success when used with growing beef cattle. Steers supplemented with cottonseed cake 3× weekly had similar BW gain during winter compared to steers supplemented daily (McIlvain and Shoop, 1962). Conversely, steers grazing winter range and supplemented with dried distillers grain daily had greater

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ADG than steers supplemented 3× weekly (Stalker et al., 2009). Therefore, the objective of our study was to evaluate the performance of young, lightweight stocker calves grazing dormant, native tallgrass pastures and supplemented protein either daily or 3× week throughout the winter.

MATERIALS AND METHODS

Animal care practices used in our study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978.1).

Angus x Hereford steer and heifer calves (n = 233; initial BW = 185 ± 28.1 kg; initial age = 162 ± 21 d) originating from the commercial cow-calf herd at Kansas State University in Manhattan, KS were used in our study. At approximately 60 d of age, males calves were surgically castrated; all calves were vaccinated against clostridial diseases (Ultrabac® 7; Pfizer Animal Health, Exton, PA) at that time and, where applicable, surgical dehorning was carried out. Following weaning in October, calves were confined to a single, dormant, native tallgrass pasture at the Kansas State University Commercial Cow-Calf Unit and were assigned randomly to 1 of 2 treatments related to protein-supplementation frequency: daily (**7X**) or thrice weekly (**3X**).

Upon separation from their dams, calves were weighed individually and given initial vaccinations against viral respiratory pathogens (Bovi-Shield Gold® 5; Pfizer Animal Health, Exton, PA) and clostridial pathogens (Ultrabac® 7; Pfizer Animal Health, Exton, PA). Calves were also given an injection of trace minerals (Multimin® 90; Multimin USA Inc., Fort Collins, CO), and treated for internal and external parasites (Dectomax® Injectable; Zoetis Inc., Kalamazoo, MI). In addition, steer calves were given a growth-promoting implant (Ralgro®; Intervet Inc., Merck Animal Health, Summit, NJ) at that time. Calves were re-vaccinated against viral respiratory pathogens and clostridial pathogens 14 d after maternal separation.

Immediately following separation from dams, calves were confined to a single earth-floor pen (minimum area = 200 m^2/calf) and allowed *ad libitum* access to native tallgrass prairie hay (88.9% DM, 8.71% CP) via 6 ring feeders (diameter = 3 m) for 4 d. Calves were released into the pasture designated for the study on the afternoon of d 4. The previously non-grazed, burned, native tallgrass pasture (130 ha) provided continual access to surface water and was stocked at 0.56 ha/calf for the duration of the study.

Pasture forage quality was estimated by clipping all plant material from within randomly-placed sampling frames (0.25 m^2; n = 2/pasture) at a height of 1 cm on 10/3, 10/31, 11/28, 1/02, 1/28, and 3/9. Samples were composited by sampling date at the conclusion of the experiment and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, and ADF. Nutrient composition was fairly consistent over the period of our study (Table 1).

TABLE 1. Nutrient composition of range forage

Sampling date	CP, % DM	NDF, % DM	ADF, % DM
10/03/2014	4.4	67.4	46.8
10/31/2014	4.1	69.9	49.6
11/28/2014	3.7	71.2	50.9
1/02/2015	3.6	72.8	51.5
1/28/2015	3.6	73.7	51.9
3/09/2015	3.9	69.1	47.6

TABLE 2. Nutrient composition of sunflower meal

Nutrient composition	
DM %	93.0
OM, % DM	25.6
CP, % DM	32.1
NDF, % DM	44.2
ADF, % DM	31.3
Ca, % DM	0.37
P, % DM	0.96

Pelleted sunflower meal (**SFM**; 93% DM, 32.1% CP), purchased from Archer Daniels Midland in Goodland, KS, was used as the supplemental CP source for our study. All calves were fed 7.0 kg of SFM weekly, with supplementation frequency depending on treatment group. Once released on pasture, calves were sorted daily into either 3X or 7X treatment groups and confined to two separate pens. Both treatments were group-fed in concrete bunks (46 cm of linear bunk space/calf). Calves assigned to 7X were fed 1.0 kg SFM/calf daily (DM basis). Calves assigned to 3X were sorted and confined in a pen daily but were supplemented with 2.3 kg SFM/calf on Monday, Wednesday, and Friday only.

Sunflower meal pellets were delivered at approximately 6-wk intervals during our study in four separate truckloads. Grab samples were collected from each truckload and frozen at -20°C. Samples were composited by weight at the conclusion of the experiment and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, OM, CP, NDF, ADF, Ca, and P (Table 2).

Calves were individually weighed at 28-d intervals over the 157 d study (Table 3). To attempt to reduce the influence of gut fill on BW, calves were penned without access to feed for 24 h before BW measurements. Calves were monitored daily for symptoms of respi-

TABLE 3. Post-weaning growth of calves supplemented with sunflower meal (SFM) either daily or thrice weekly while grazing dormant native tallgrass range during winter

Item	7X[1]	3X[2]	SEM	*P*-value
Weaning BW, kg	183	186	3.7	0.42
BW on d 28, kg	193	197	3.9	0.31
BW on d 56, kg	201	205	4.3	0.44
BW on d 91, kg	201	205	4.2	0.33
BW on d 117, kg	206	207	4.2	0.84
BW on d 157, kg	211	212	4.2	0.68
BW change 0 to 157 d, kg	27.0	25.6	1.98	0.49
ADG d 0 to 28, kg	0.33	0.37	0.035	0.28
ADG d 29 to 56, kg	0.29	0.27	0.039	0.70
ADG d 57 to 91, kg	0.00	0.01	0.034	0.64
ADG d 92 to 117, kg	0.20	0.07	0.037	< 0.01
ADG d 118 to 157, kg	0.11	0.14	0.019	0.22
ADG d 0 to 157, kg	0.17	0.16	0.013	0.48

[1]Calves were supplemented with 1.0 kg SFM (DM basis) daily for 157 d.

[2]Calves were supplemented with 2.3 kg SFM (DM basis) thrice weekly for 157 d.

ratory disease and conjunctivitis. Calves with clinical signs of BRD, as judged by animal caretakers, were removed from pastures and evaluated. Calves were assigned a clinical-illness score (scale: 1 to 4; 1 = normal, 4 = moribund), weighed, and assessed for febrile response. Calves with a clinical illness score > 1 and a rectal temperature > 40.0°C were treated with therapeutic antibiotics according to label directions (first incidence = Baytril®, Bayer Animal Health, Shawnee Mission, KS; second incidence = Resflor Gold®, Merck Animal Health, Summit, NJ). Calves were evaluated 72 h following treatment and re-treated if clinical signs of BRD persisted. Calves showing signs of conjunctivitis (i.e., pinkeye) were treated using oxytetracycline (LA 200®; Zoetis Inc., Kalamazoo, MI). Calves were evaluated 14 d following treatment and re-treated if clinical signs of conjunctivitis persisted.

Growth performance was analyzed as a mixed model with a 1-way treatment structure as a completely-randomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). Animal was the experimental unit. The model statement included a term for the fixed effect of treatment. When protected by a significant *F*-test ($P \leq 0.05$), least-squares treatment means were separated using the method of Least Significant Difference. Means were considered different when $P \leq 0.05$.

RESULTS AND DISCUSSION

Calf BW was not different ($P \geq 0.31$) between treatments at any time during the study. Likewise, calf BW change over the course of the study was not influenced ($P = 0.49$) by supplementation frequency. Calf ADG was not different ($P \geq 0.22$) between treatments from d 0 to 28, d 29 to 56, d 57 to 91, or d 118 to 157; moreover, ADG from d 0 to 157 was not different ($P = 0.48$) between treatments. For a brief period between d 92 to d 117, calves assigned to 7X had greater ($P < 0.01$) ADG than calves assigned to 3X; however, this result was inconsequential to overall ADG. Calf BW changes during our study were modest but typical of winter grazing operations in the tallgrass prairie region of Kansas. Poor forage quality likely limited performance.

Dormant-season grazing with calves is common for ranchers in the tallgrass prairie region of Kansas. Calves are purchased in the late fall when seasonal price discounts are relatively high and grown at modest rates on dormant, native tallgrass range until spring. From approximately April 15 to July 15, calves then graze actively-growing native tallgrass range and can achieve ADG that exceed 1 kg. Although winter BW gains are modest in this system, subsequent summer BW gains are thought to offset poor winter performance.

The contracted price of SFM at the initiation of our study was $235.38/ton; feed cost per calf was estimated at $39.67 for the 157-d period of our study (i.e., 1.1 kg SFM × 157 d × $0.235/kg; as-fed basis). Feed delivery cost for 7X was estimated at $39.25/calf (i.e., 157 d × $0.25/calf), whereas feed delivery cost for 3X was only $16.25/calf for the 157-d period (i.e., 65 d × $0.25/calf).

IMPLICATIONS

Daily protein supplementation did not improve growth performance relative to thrice-weekly protein supplementation when weekly CP delivery was held constant between treatments. Supplementing CP to stocker calves trice weekly saved 59% ($23.00/calf) in feed- delivery cost throughout the winter compared with daily CP supplementation. Stalker et al. (2009) reported that steers supplemented 3× weekly had 55% lesser supplement delivery costs than steers supplemented daily.

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The effects of AC Saltlander seeding rate on forage production and foxtail barley weed suppression on saline soils[1]

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ABSTRACT: Soil salinity is one of major issues that reduced agricultural production across the world. A feasible solution is to breed or introduce salt tolerant forage varieties. AC Saltlander green wheatgrass (Elymus hoffmannii) (ACS) was developed at Agriculture and Agri-Food Canada - Swift Current Research and Development Centre (AAFC-SCRDC) to tolerate root-zone salinity. The objective of the present study was to evaluate the effects of different ACS seeding rates on forage production and ability to suppress foxtail barley (Hordeum jubatum) (FTB) grown on saline soils. A consecutive 4-yr field experiment was carried out on a moderate (8 dS/ m < EC_e < 16 dS/ m) to severe (ECe > 16 dS/ m) saline site at AAFC-SCRDC from 2013 to 2016. A completely randomized block design was used with 4 seeding rate treatments as follows: 2.8 kg/ha, 5.6 kg/ha, 11.2 kg/ha, and 16.8 kg/ha. All interactions were not significant ($P > 0.05$), thus only main effects were presented. Results showed that: AC Saltlander mean forage DM yields (2013-2015) were 638.8 ± 57.0, 773.4 ± 48.0, 749.2 ± 46.1 and 796.2 ± 46.6 kg/ha, respectively with increasing seeding rates. AC Saltlander yield on 2.8 kg/ ha was lower ($P = 0.04$) than the 16.8kg/ ha seeding rate and no differences ($P > 0.05$) were found among the top three seeding rates. No differences ($P > 0.05$) in FTB forage yields with seeding rates were observed. Neither ACS nor FTB forage yields among different seeding rates showed any statistical differences ($P > 0.05$) in each year from 2013 to 2015. However, ACS yields in 2015 were observed lower ($P < 0.0001$) than the other two years. AC Saltlander mean relative yield was the lowest (67.2%) while FTB relative yield was the highest (32.8%) at 2.8 kg/ha ACS seeding rate ($P = 0.007$). No differences ($P > 0.05$) for ACS and FTB were found among the other three treatments where ACS relative yields were 79.7%, 78.6% and 81.0% as seeding rates increased, and FTB forage yields only accounted for around 19-33% of the total forage yields. Results showed no benefits in ACS forage production or FTB suppression from increasing seeding rates to the top two rates. The best seeding rate to achieve good ACS yield and FTB weed control was observed at 5.6 kg/ ha. Since seed costs for ACS are high, using the most effective seeding rate can obtain cost benefits.

Key words: forage yield, green wheatgrass, seeding rate, soil salinity, weed suppression
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INTRODUCTION

Soil salinity is considered to be the second largest cause of losses from agricultural production (Wiebe et al., 2007; Huang et al., 2015). Plant establishment, growth and productivity are limited by excessive salinity (Rengasamy, 2010; Munns and Gilliham, 2015). more than 800 million hectare lands (over 6% total land area) are salt affected throughout the world (Munns, 2005; Rengasamy, 2010; Anderson et al., 2015). It was estimated that 4 million hectare (10%) of cultivated lands within Canadian prairies are suffering salinization and caused around $250 million in economic losses each year and even increasing by at least $1 million annually (Dumanski et al., 1986; Wiebe et al.,

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2007; Anderson et al., 2015). there are roughly 1.6 million hectare of saline soil in Saskatchewan, Canada (Hangs et al., 2011).

Agronomic and engineering solutions for soil salinity are inefficient up to now and a feasible way is to introduce and breed greater salt tolerant forage varieties (Munns and Gilliham, 2015). AC Saltlander (*Elymus hoffmannii*) (ACS) is the first cultivar of green wheatgrass developed at Agriculture and Agri-Food Canada - Swift Current Research and Development Centre (AAFC-SCRDC) to tolerate root-zone salinity. It turns green in the early spring and is palatable for both grazing and haying with high yield and nutrient value (Steppuhn and Asay, 2005; Steppuhn et al., 2006). nonetheless, the seed yields of AC Saltlander is low partly due to the creeping root growth, hybridity and sensitive to the temperature and humidity change during pollination period, therefore, the seed prices are high. Higher seeding rate may inevitably increase production cost, whereas lower seeding rate may increase the risks for reducing production. The optimum seeding rate is key to profit maximization. Seeding rate strongly impacts the plant to make use of resources like light, water and nutrients (Arduini et al., 2006). however, yield followed a positive, negative or even unchanged response as seeding rate increased, which depends on the species and physical conditions (Bhatta et al., 2017).

Seeding rate is an effective non-chemical tool for reducing weed costs and herbicide uses (Lemerle et al., 2016). Foxtail barley (*Hordeum jubatum*) (FTB), is a severe disturbance weed with high dispersibility and competitivity but unpalatability in prairie, which has been proven not easy to control especially in saline soil. AC Saltlander grows well in saline soil and has a potential to suppress FTB at all salinity levels (Steppuhn and Asay, 2005). The objective of the present study was to evaluate the effects of different ACS seeding rates on forage production and ability to suppress FTB grown on saline soil conditions.

MATERIALS AND METHODS

Experimental design and treatments

The experiment was conducted at the AAFC-SCRDC North Farm site (50°17′50″ N, 107°45′16″ W, 817 m elevation) from 2011 to 2015. The soil was sandy-loam textured and classified as a Haverhill association. The site was identified as a moderate (8 dS/ m < ECe < 16 dS/ m) to severe (ECe > 16 dS/ m) salinity and was staked for plot layout, and each plot was 12.19 m in length and 1.83 m wide. A completely randomized block design was used with 4 seeding rate treatments as follows: 2.8 kg/ ha, 5.6 kg/ ha, 11.2 kg/ ha, and 16.8 kg/ ha. Each treatment had 4 replications (Fig. 1).

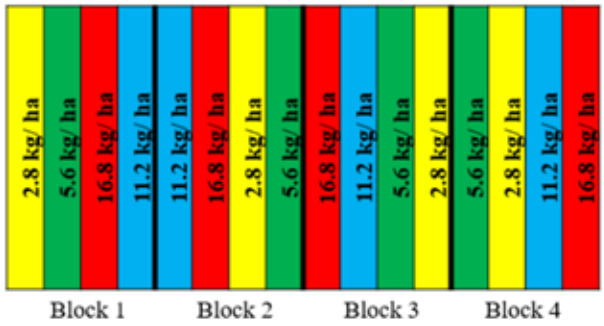

Figure 1. Schematic diagram for experimental plots and blocks

Seeding and sample collection

AC Saltlander were seeded in June 16th, 2011 after an application of glyphosate (5 L/ ha), cultivation and harrow packed. A disc-drill forage plot-seeder with 6 discs spaced 30.5 cm apart was used to sow in each plot. To control the *Kochia*, 2, 4-D was applied at 0.95 L/ ha on June 1st, 2012. Four quadrats (1 m2) were randomly located in each plot to harvest the yield in the middle of july from 2013 to 2015 production years. AC Saltlander and FTB were clipped by scissors flush with the ground and bagged separately. All samples were oven-dried at 65 °C by 48 h to constant weight and then weighted.

Statistical Analysis

Data were analyzed as a completely randomized block design using R software for windows (version 3.3.2). Normality and variance homogeneity of samples were determined by the Shapiro-Wilk test and the Barlett test respectively. Models for ACS and FTB included the effects of seeding rate, year, seeding rate and year interaction were conducted on biomass production and proportional relative yield. Significance was declared at $P < 0.05$. The tukey *t*-test was used to compare differences between multiple treatments. Relative yield of ACS (%) = yield of ACS / total yield ×100; Relative yield of FTB (%) = yield of FTB / total yield ×100.

RESULTS AND DISSCUSSION

Weather

The annual long term mean (LTM) precipitation and temperature from 1983 to 2016 (34-yr) were 383.9 mm and 4.3°C, respectively. From 2011 to 2015, the annual mean precipitations were 457.4, 406.0, 376.5, 455.5 and 356.2 mm, as well as, the annual mean temperatures were 3.7, 5.1, 3.1, 3.2 and 5.5 °C, respectively. Figure 2 shows the monthly mean precipitations and air temperatures.

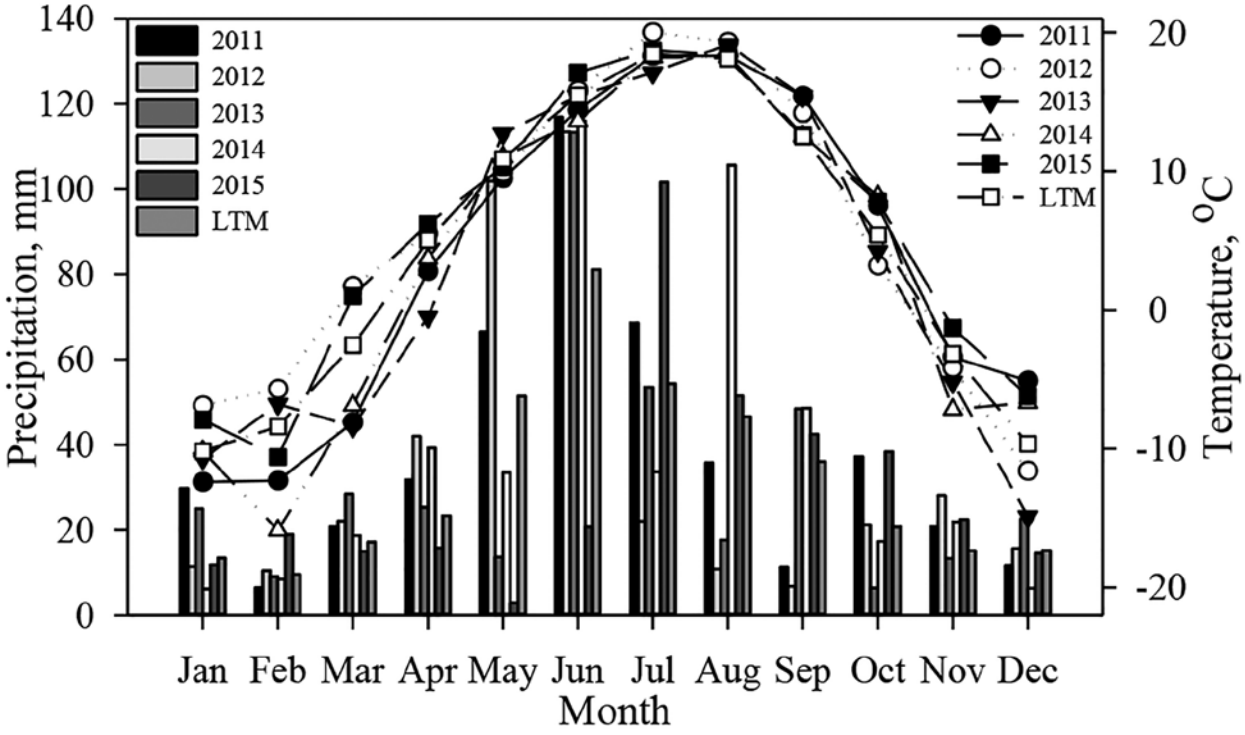

Figure 2. Monthly mean precipitations and air temperatures

Yields of ACS and FTB

All interactions were not significant ($P > 0.05$), thus only main effects were presented. AC Saltlander mean forage DM yields (2013-2015) were 638.8 ± 57.0, 773.4 ± 48.0, 749.2 ± 46.1 and 796.2 ± 46.6 kg/ha respectively with increasing seeding rates. AC Saltlander yield on 2.8 kg/ ha was lower ($P = 0.04$) than 16.8kg/ ha seeding rate and no differences ($P > 0.05$) were found among the top three seeding rates. No differences ($P > 0.05$) in FTB forage yields with seeding rates were observed (Fig. 3). AC Saltlander propagates more ramets through rhizomes to maintain high forage production due to more nutrients and less competitions at low seeding rates (Steppuhn et al., 2006). Lower seeding rates have an adversely effect on forage production, but at higher seeding rates the intense competitions among interspecies and intraspecies may limit forage growth and production (Arduini et al., 2006).

Both ACS and FTB forage yield were differences ($P < 0.0001$) among years. AC Saltlander yields in 2015 were observed lower ($P < 0.0001$) than the other two years (Fig. 4). Foxtail barley yields were highest in 2013 and lowest in 2015. The growth and production of ACS were more related to interannual variability and annual distribution of the precipitation. Low precipitation and higher growing temperature during the 2015 spring period reduced ACS production (Fig. 2).

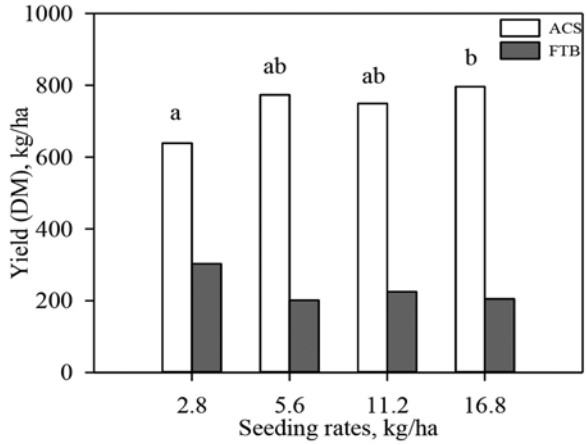

Figure 3. Mean yields (2013-2015) of AC Saltlander (ACS) and foxtail barley (FTB) under different seeding rates.

Relative yields of ACS and FTB

AC Saltlander mean relative yield was the lowest (67.2%) while FTB relative yield was the highest (32.8%) at 2.8 kg/ha ACS seeding rate ($P = 0.007$). No differences ($P > 0.05$) for ACS and FTB were found among the other three treatments where ACS relative yields were 79.7%, 78.6% and 81.0% as seeding rate increased, and FTB forage yields only accounted for around 19-33% of the total forage yields (Fig. 5).

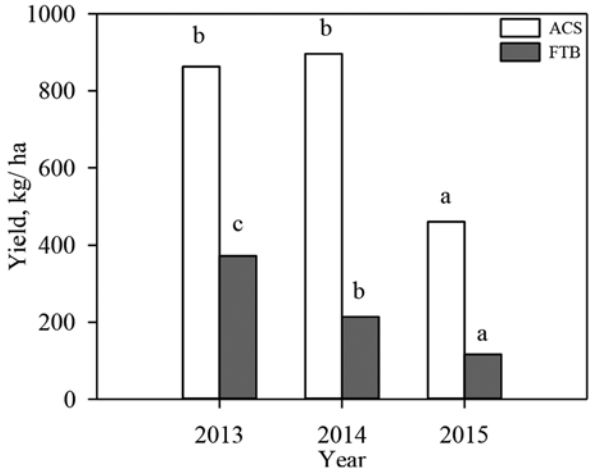

Figure 4. Yields of AC Saltlander (ACS) under different seeding rates from 2013 to 2015

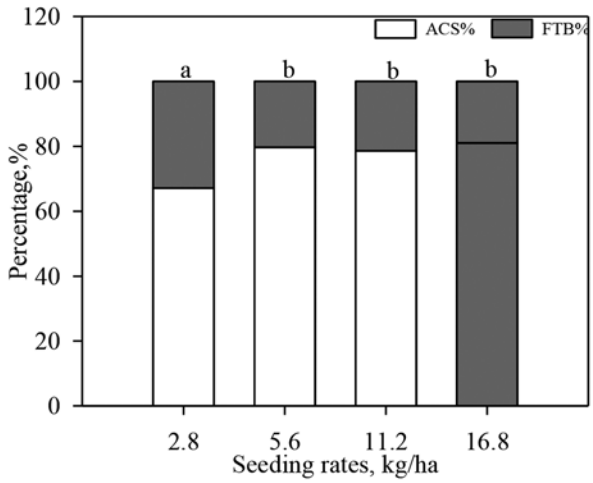

Figure 5. Mean relative yields (2013-2015) of AC Saltlander (ACS) and foxtail barley (FTB)

IMPLICATIONS

Study results showed no benefits in ACS forage production or FTB suppression from increasing seeding rates to the top two rates. The best seeding rate to achieve good ACS yield and FTB weed control was observed at 5.6 kg/ ha. Since seed costs for ACS are high, using the most effective seeding rate can obtain cost benefits. Further research is needed to evaluate ACS forage performance and FTB suppression over longer production years (> 5) and different environments and soil conditions need to be considered as well.

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Corpora lutea in superovulated ewes: Cell proliferation, vascularity, and protein expression of endothelial nitric oxide synthase and soluble guanylyl cyclase[1]

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ABSTRACT: The corpora lutea (CL) is an ovarian structure critical for maintenance of reproductive cyclicity and pregnancy. Diet and/or diet components may affect some luteal functions. FSH is widely used to induce multiple follicle development and superovulation. We hypothesized that FSH will affect luteal function in ewes fed different planes of nutrition. The aim was to determine FSH-treatment effects on ovulation, CL growth, cell proliferation, vascularity, expression of eNOS and sGC proteins, and luteal and serum progesterone (P4) concentration in control (C), overfed (O) and underfed (U) ewes. Ewes (n = 34) were randomly assigned into one of three different nutrition groups: C (fed 2.14 Mcal/kg), O (2 x C), and U (0.6 x C). Nutritional treatment was initiated 60 days before d 0 of the estrous cycle. Ewes were injected with FSH on d 13-15 of the estrous cycle, and blood samples and ovaries were collected at the early- and mid-luteal phases. Luteal expression of Ki67 (proliferating cell marker), CD31 (endothelial cell marker), and eNOS and sGC was determined using immunohistochemistry and image analysis. The CL number tended ($P = 0.2$) to be less in U than C or O (9.6 ± 1.9 vs. 13.4 ± 1.7 and 13.8 ± 2.3). CL weights tended to be less ($P < 0.08$) at the early- than mid-luteal phase (307 ± 28 vs. 385 ± 25 mg). Cell proliferation (18.2 ± 0.7 vs. 5.4 ± 0.5 %), vascularity (CD31 positive area; 8.5 ± 0.6 vs. 4.8 ± 0.6 %), and eNOS (16 ± 0.5 vs. 13 ± 0.5 %) but not sGC expression were greater ($P < 0.001$) at the early- than mid-luteal phase. Serum (8 ± 1 vs. 10.3 ± 1.3 ng/ml), but not luteal tissue P4 concentrations tended to be lower ($P = 0.09$) at the early- than mid-luteal phase. Thus, luteal cell proliferation and vascularity, and expression of eNOS and sGC depend on the stage of luteal development, but not diet, in FSH-treated ewes. The mechanisms of FSH effects on luteal function remain to be elucidated.

Key words: corpora lutea, endothelial nitric oxide synthase, ewe, follicle-stimulating hormone, soluble guanylyl cyclase, vascularity
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INTRODUCTION

The corpus luteum (CL), the major source of progesterone (P4) in females, is a transient endocrine gland, which grows, differentiates and regresses during each estrous cycle or pregnancy, and plays a major role in the reproductive processes (Jablonka-Shariff et al., 1993; Grazul-Bilska et al., 1997; Reynolds et al., 1994, 2000; Niswender and Nett, 1994; Stouffer and Hennebold, 2015). The CL growth, differentiation, and regression depend on a balance between luteotropic (e.g., LH) and luteolytic (e.g., prostaglandin $F_{2\alpha}$) factors that also regulate P4 secretion (Niswender and Nett, 1994; Stouffer and Hennebold, 2015).

Dynamic changes within CL during each estrous cycle include cell proliferation, and establishment and growth of vascular bed during early- and mid-luteal phases of the estrous cycle (Bass et al., 2017). The CL consists of several cell types including parenchymal steroidogenic small and large lu-

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teal cells, vascular cells (endothelial cells, pericytes and smooth muscle cells), fibroblasts, immune cells, and others (Niswender and Nett, 1994; Stouffer and Hennebold., 2015). It has been suggested that these cell types interact to maintain normal luteal function (Grazul-Bilska et al., 1997; Stouffer and Hennebold, 2015). The CL is highly vascularized, and vascular cells comprise more than 50% of the total cells in the CL (Reynolds et al., 1994). Vascular growth and function is regulated by several angiogenic and growth factors including those associated with production of nitric oxide (NO; Reynolds et al., 1994, 2000; Bass et al., 2017). The NO is a free-radical gaseous molecule that is produced in endothelial cells by endothelial NO synthase (eNOS)-mediated breakdown of L-arginine (Beckman et al., 2006). In addition, NO diffuses rapidly from the endothelial cells into the underlying smooth muscle cells or pericytes where it activates soluble guanylate cyclase (sGC). Both, eNOS and sGC are expressed in the CL of non-pregnant sheep (Bass et al., 2017).

Follicle stimulating hormone has been widely used to stimulate multiple follicle development and/or superovulation in mammalian species (Cognie, 1999; Grazul-Bilska et al., 2007). After FSH-treatment, the number of CL and serum P4 are greater than in non-treated sheep (Stormshak et al., 1963; McClellan et al., 1975; Grazul-Bilska et al., 2007), but the morphology of the CL was similar for superovulated and non-superovulated ewes (McClellan et al., 1975; Hild-Petito et al., 1987). The aim of this study was to characterize the CL from superovulated ewes by determining cell proliferation, vascularity (marked by CD31 expression), expression of eNOS and sGC proteins, and serum and luteal P4 concentration at the early- and mid-luteal stages of the estrous cycle. These stages correspond to rapid growth and differentiating phases of luteal development (Jablonka-Shariff et al., 1993; Grazul-Bilska et al., 1997).

MATERIALS AND METHODS

Animals and Experimental Design

All animal procedures performed were approved by the North Dakota State University (NDSU) Institutional Animal Care and Use Committee (#A12013). The study was initiated during the normal breeding season in August and finished in December. Non-pregnant, non-lactating Rambouillet ewes between 3 to 5 years of age and of similar genetic background were individually penned at the Animal Nutrition and Physiology Center on the NDSU. Ewes were stratified by BW and randomly assigned into one of three dietary groups: maintenance-control (C; 100% National Research Council [NRC] requirements; 2.4 Mcal of metabolizable energy [ME]/kg BW), overfed (O; 200% NRC requirements), or underfed (U; 60% NRC requirements), as previously described (Kaminski et al., 2015; Bass et al., 2017). Diets were initiated 60 days prior to the onset of estrus (day 0). Ewes were fed their individual diets twice daily at 0800 and 1500 h for the duration of the experiment, and ewes were weighed once weekly. Individual rations were adjusted weekly to ensure the proper BW (e.g., C, O, and U) was achieved at day 0 and maintained throughout the estrous cycle and until completion of the experiment. Estrus was synchronized by insertion of a controlled internal drug release (CIDR) device for 14 days. Ewes (n=34) were injected with FSH-P (Sioux Biochemical, Sioux Center, IA, USA) as described (Grazul-Bilska et al., 2017a). Initial BW and BCS were similar for all groups (55.9±1.5 kg and 2.9±0.1, respectively). Throughout the study, C (n=11) maintained BW, O (n=11) ewes gained ($P<0.001$) 13.1±1 kg and BCS increased by 1.0±0.1, and U (n=12) ewes lost ($P<0.001$) 7.7±1 kg and BCS decreased by 0.7±0.1, similar to our previous studies (Grazul-Bilska et al., 2012, 2017a; Kaminski et al., 2015; Bass et al., 2017).

Tissue and Blood Collection, Immunohistochemistry, Image Analysis and Progesterone Analysis

Serum and ovaries were collected at the early- and mid-luteal phases of the estrous cycle (Grazul-Bilska et al., 2017a). The number of CL was recorded, and CLs were dissected from the ovaries and weighed. Randomly selected CL from each ewe were used for immuno-histochemistry and evaluation of P4 concentrations.

Immunohistochemistry was performed as described (Bass et al., 2017). Tissue sections underwent antigen recovery, and then were washed, blocked, and incubated with a primary antibody against Ki67 (mouse monoclonal, Vector Labs, Burlingame, CA), CD31 (rabbit polyclonal; Abcam Biotech Company, San Francisco, CA), eNOS (mouse monoclonal; BD Biosciences, San Jose, CA) or sGC (rabbit polyclonal; Cayman Chemical, Ann Arbor, MI), and then with a secondary antibody conjugated with a fluorescent marker. Photomicrographs were taken with a Zeiss Imager M2 epifluorescence microscope (Zeiss Inc., Thornwood, NY, USA). The percentage of area that exhibited positive staining for CD31, eNOS and sGC was evaluated quantitatively with an image analysis system (Image Pro-Plus, Media Cybernetics, Silver Spring, MD; Bass et al., 2017). The labeling index (LI) was calculated as the percentage of proliferating Ki67-positive cells out of the total number of cells within tissue area. For each CL, four randomly chosen fields were evaluated. Background fluorescence

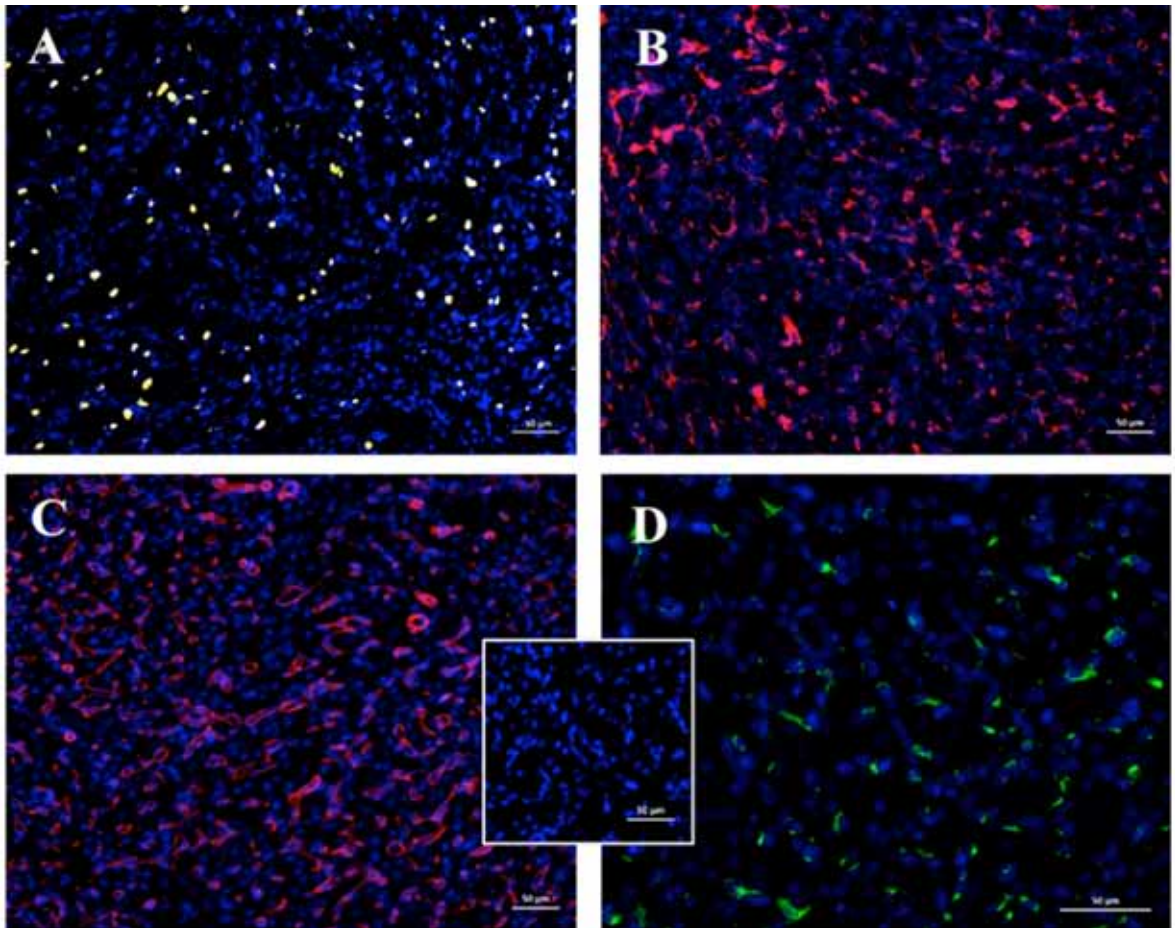

Figure 1. Representative images of immunofluorescent staining of Ki67 (yellow; A), CD31 (red; B), eNOS (red; C) and sGC (green; D) in luteal tissues from the early-luteal phase of the estrous cycle. Blue color in each image indicates DAPI nuclei staining. Control staining where primary antibody was omitted is in inset in C and D. Note expression of CD31, eNOS, and sGC in blood vessels. Size bar for all images = 50 µm.

was minimal and was adjusted to the same level for each section by the image analysis system.

Progesterone concentration in serum and luteal tissues was determined using a solid phase chemiluminescence, competitive binding immunoassay (Immulite 1000, Siemens, PA, USA), as previously described (Kaminski et al., 2015; Bass et al., 2017). Each sample was run in duplicate. The intra-assay CV was 6.1% for serum and 5.5% for luteal P4.

Statistical analysis

Data were analyzed statistically using the GLM procedure of SAS 9.2 (Cary, NC, USA). When the F-test was significant ($P \leq 0.05$), difference between specific means were separated using least significant difference.

RESULTS

Weights of CL and serum P4 concentrations were greater ($P < 0.02$-0.09) at mid- than early-luteal phase (210 ± 17 vs. 336 ± 22g, and 8.0 ± 1.0 vs. 10.3 ± 1.3 ng/ml, respectively) and were not affected by nutritional plane. Concentration of P4 in luteal tissues (8.7 ± 0.8 mg/g tissues) was similar at the early- and mid -luteal phases.

The number of CL, and thus ovulation rates, was greater ($P < 0.03$) in O and C than U (13.4 ± 1.6 and 13.7 ± 2.3 vs. 9.6 ± 1.8). The number of CL ranged 4-29, 4-27, and 4-22 in C, O, and U, respectively. The proportion of ewes that did not respond to FSH treatment (the number of CL ≤3) was 21, 7 and 27% in C, O and U, respectively. In addition, 79, 71, and 60% of C, O, and U had more than 5 ovulations, respectively.

Ki67, CD31, eNOS, and sGC proteins were immunodetected in the CL from early- and mid-luteal phases (Fig. 1). Ki67 (Fig. 1A) was localized to cell nuclei, but CD31 (Fig. 1B), eNOS (Fig. 1C) and sGC (Fig. 1D) to cytoplasm of luteal vascular cells. Labeling index (LI) and expression of CD31 and eNOS were greater ($P < 0.001$) at the early- than mid-luteal phase, and plane of nutrition did not affect any of these measurements; therefore, combined data are presented (Fig. 2). Expression of sGC protein was similar at the early- and mid-luteal phases of the estrous cycle, and was not affected by nutritional plane (Fig. 1).

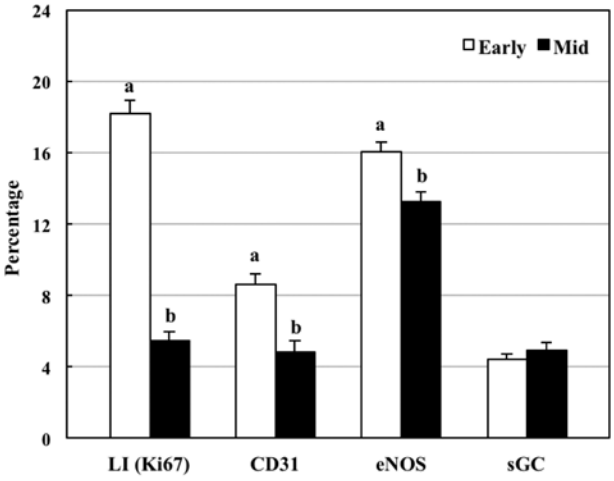

Figure 2. Labeling index (LI; based on Ki67 staining), and expression of CD31, eNOS and sGC in the CL from early- and mid- luteal phases of the estrous cycle. $^{a,b}P < 0.0001$-0.0006, means ± SEM with different superscripts differ at the early- vs. mid-luteal phase within a specific measurement.

DISCUSSION

The present experiment demonstrated that diet affected the number of ovulations, but did not affect several measurements of luteal functions. However, CL weight, cell proliferation, vascularity, expression of eNOS proteins, and serum P4 concentration were affected by the phase of the estrous cycle. Luteal P4 concentration and sGC expression and were not affected by the phase estrous cycle or diet in superovulated ewes.

In the present study, the mean number of CL/ewe varied from 9 to 14 depending on the nutritional plane. Similarly, in our numerous previous studies the number of CL in superovulated ewes fed maintenance diet varied from 12 to 20 (Grazul-Bilska et al., 2007). Furthermore, the number of CL in superovulated U was less than in C or O ewes, similar to non-superovulated ewes (Bass et al., 2017). The mechanisms of nutritional effects on ovulation remain to be elucidated.

Similar to previous reports, in this study, Ki67 was immunolocalized to cell nuclei but CD31, eNOS and sGC proteins were detected in vascular cells in the ovine CL demonstrating comparable pattern of protein distribution in superovulated and non-superovulated ewes (Al-Gubory et al., 2005; Grazul-Bilska et al., 2006; Bass et al., 2017). Others have demonstrated that luteal morphology, responsiveness of luteal cells to LH and dbcAMP, and the ratio of small to large luteal cells were similar in superovulated and non-superovulated ewes (McClellan et al., 1975; Hild-Petito et al., 1987). For superovulated ewes in this study, from the early- to mid-luteal phase, the pattern of changes of several measurements of luteal functions including CL weight, cell proliferation, vascularity, and serum P4 concentration, were similar to those observed in non-superovulated ewes (Jablonka-Shariff et al., 1993; Grazul-Bilska et al., 2007; Bass et al., 2017). For example, greater proliferation rates and vascularity at the early- than mid-luteal phases have been reported for several species including sheep, cows, and primates (Jablonka-Shariff et al., 1993; Fraser et al., 2000; Young et al., 2000; Hünigen et al., 2008; Yoshioka et al., 2013; Bass et al., 2017). Expression of eNOS in this study was greater at the early- than mid-luteal phase similar to reported before for non-superovulated ewes (Grazul-Bilska et al., 2006). In addition, pattern of sGC protein expression was similar in both superovulated and non-superovulated ewes (Bass et al., 2017). Thus, these results indicate several similarities in luteal functions in superovulated and non-superovulated ewes, emphasizing that superovulated model can be used to study luteal functions in sheep.

In this study, serum P4 concentration was ~5-fold greater that reported for non-superovulated sheep (Jablonka-Shariff et al., 1993; Grazul-Bilska et al., 2007). The greater P4 concentration in superovulated animals is due to the enhanced number of CL, which consequently produce more P4 (Grazul-Bilska et al., 2007). However, the luteal P4 concentration was similar in superovulated vs. non-superovulated ewes indicating that production of P4/luteal tissue unit is similar.

In this study, diet did not affect any measurements of luteal functions. In our previous study, diet also did not affect serum and luteal P4 concentration in non-superovulated sheep (Bass et al., 2017). However, vascularity measured by CD31 expression was reduced at the early luteal phase in O and U ewes, and mid-luteal phase in O ewes, and cell proliferation was enhanced in O ewes at the early luteal phase compared to C group in non-superovulated sheep (Bass et al., 2017). These differences are likely due to the effects of FSH-treatment on luteal functions. Currently, the mechanism of FSH-treatment effects on luteal cells is unknown. However, FSH can likely exert direct effects on the CL since FSHR are expressed in luteal tissues, and the level of expression differs at the early- and mid-luteal phases (Grazul-Bilska et al., 2017b). Therefore, we postulate that FSH may play different role on regulation of luteal functions depending on the stage of luteal development. However, this hypothesis remains to be tested.

Implications

This study demonstrated that diet affected ovulation rates in superovulated ewes but not several measurements of luteal function. From early- to mid-luteal phase, pattern of changes in cell proliferation, vascularity, expression of eNOS and sGC, and serum

P4 concentration is similar in superovulated and non-superovulated ewes. However, CL weight at the mid-luteal phase, the rates of cell proliferation, vascularity, and expression levels of eNOS and sGC proteins, and serum P4 concentration at the early- and mid-luteal phases differ in superovulated and non-superovulated ewes. Furthermore, luteal P4 concentration is similar in superovulated and non-superovulated ewes, indicating that luteal cells produce P4 at the similar rates in both groups. Since basic function of the CL which is the rate of production of P4/tissue unit is not affected by FSH treatment, and cells from superovulated animals respond to the LH-treatment (Grazul-Bilska et al., 1991, 1995, 1996a,b; Hild-Petito et al., 1987), we postulate that the superovulation model is a valid and reliable means for studying luteal functions.

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Changes in temporal concentrations of fibroblast growth factor 21 in beef heifers[1]

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ABSTRACT: The secretion of Fibroblast Growth Factor 21 (FGF21) in peripubertal beef heifers has yet to be characterized. Herein, we investigated the secretory pattern and the potential role of FGF21 in regulating onset of puberty in beef heifers. Heifers (n = 33) were removed from pasture, housed in a drylot fitted with GrowSafe feed bunks, and fed a total mixed ration to meet or exceed NRC requirements. Body weights were recorded and blood samples collected on d -7, 0, 28, and 56 of the experiment. Samples were assayed for circulating concentrations of FGF21, progesterone, and cortisol. Individual ADG, residual feed intake (RFI), and back fat thickness (BF) were determined on d 56. Reproductive status was determined utilizing transrectal ultrasonography and circulating concentrations of progesterone. Serum concentrations of FGF21 increased ($P < 0.0001$) from d -7 (411.41 ± 63.45 pg/mL) to d 0 (817.28 ± 63.45 pg/mL). By d 56 (497.62 ± 63.45 pg/mL), concentrations of FGF21 were not different ($P = 0.29$) from concentrations observed on d -7. When serum concentrations of FGF21 were analyzed by RFI rank, Low RFI heifers (701.77 ± 77.59 pg/mL) had greater ($P = 0.03$) concentrations of FGF21 than High RFI heifers (402.93 ± 105.73 pg/mL). However, neither groups differed ($P \geq 0.15$) from Mid RFI heifers (595.88 ± 77.59 pg/mL). We were also able to observed a correlation ($y = -0.0002x + 0.5022$; $R^2 = 0.28$) between elevated serum concentrations of FGF21 on d 56 with reduced ($P = 0.002$) ADG. Concentrations of cortisol tended ($P = 0.08$) to increase during the experimental period, while concentrations of progesterone remained the same ($P = 0.24$). However, we were not able to demonstrate a difference ($P = 0.12$) in circulating concentrations of FGF21 between ovulatory and non-ovulatory heifers. These data indicate that FGF21 is not a useful biomarker to indicate onset of puberty in beef heifers. However, circulating concentrations of FGF21 may be a suitable indicator of nutritional status and performance in beef heifers.

Key words: fibroblast growth factor 21, heifer, performance, puberty, residual feed intake
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INTRODUCTION

The extensive use of grazing systems for beef cattle and the high variation in forage quality throughout the year has a large impact on production. This variation in grazing forage quality and availability has driven the utilization of harvested forage and dietary supplementation. Alterations in dietary supply alter the nutritional and physiological status of animals (Baldwin and Bywater, 1984). To maintain production sustainability it is important to consider the impact that nutrient restriction has on reproductive efficiency of females (Hess et al., 2005). Therefore, it is critical to understand how females adapt to dietary changes throughout development and as feeding regimens change.

It is theorized that a peptide synthesized and secreted by the liver (Fibroblast Growth Factor 21; **FGF21**) impacts energy metabolism and reproductive function (Inagaki et al., 2007; Owen et al., 2013). Secretion of FGF21 is dependent on dietary intake and stage of production in dairy cattle (Khan et al., 2014). Furthermore, transgenic female mice that overexpress FGF21 exhibit abnormal estrous cycles and infertility (Inagaki et al., 2007). This infertility is driven by an inability of the hypothalamus to elicit an appropriate GnRH signal to the pituitary in response to estradiol, thus delaying or preventing the onset of puberty (Owen et al., 2013). Therefore, elevated circulating con-

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centrations of FGF21 may play a role in regulating the pubertal onset of estrous cyclicity in malnourished females.

In prepubertal heifers, expression of the FGF receptors was observed in the liver, subcutaneous adipose, and perirenal adipose tissue (Schoenberg et al., 2011). However, expression of the FGF receptors in these tissues was not evaluated once heifers attained puberty. Further investigation is also needed in order to establish the physiological function of peripheral concentrations of FGF21 during the peripubertal period.

The present study aimed to investigate the temporal associations between concentrations of FGF21, progesterone, and cortisol with ADG and residual feed intake (**RFI**). Our laboratory has demonstrated the dramatic increase in circulating concentrations of FGF21 during late gestation and early lactation in beef cows (Prezotto et al., 2016). However, no studies have associated elevated concentrations of FGF21 with performance, stress, and onset of puberty in beef heifers. Based on previous research, we hypothesized that: 1) circulating concentrations of FGF21 are increased when heifers transition from a grazing forage system to harvested forage offered in a drylot, 2) circulating concentrations of FGF21 decrease prior to the onset of puberty, and 3) reduced animal performance is associated with elevated circulating concentrations of FGF21.

MATERIALS AND METHODS

Animals and experimental design

Animal care and use protocols were approved by the Montana State University Agricultural Animal Care and Use Committee. The objectives of this experiment were to characterize the secretion of FGF21 in beef heifers during the peripubertal period and correlate these concentrations with the onset of puberty, concentration of cortisol, RFI, ADG, and back fat thickness (**BF**). To achieve these objectives, 33 Angus heifers (8.0 ± 0.1 mo of age; 273.4 ± 24.0 kg) were utilized. Heifers were removed from pasture and housed in a drylot fitted with a GrowSafe feeding system for 70 d to determine individual intake and calculate ADG and RFI as previously described (Koch et al., 1963). While in the drylot, heifers were offered a total mixed ration (Table 1) to meet or exceed NRC requirements. Body weights were recorded on d -7, 0, 28, and 56 of the experiment to ensure heifers gained 0.45 kg per d.

TABLE 1. Dietary ingredients and composition of TMR

Item	%
Ingredient, % of DM	
Corn silage	34.8
Oat straw	33.2
Mixed grass hay	32.0
Diet composition, % of DM	
Crude protein	7.20
Total digestible nutrients	56.7
Effective neutral detergent fiber	47.6
Net energy for maintenance, MJ/kg	5.33
Net energy for gain, MJ/kg	2.67

Harvest of serum

Heifers were fasted for 16 h and blood samples collected via jugular venipuncture on d -7, 0, 28, and 56. Blood samples were collected into tubes containing no additive, placed immediately on ice for 30 min, and then set at room temperature for 1 h. Once clotted, samples were centrifuged (2,500 × G for 15 min). Serum was harvested and stored (-20ºC) until assayed for concentrations of FGF21, progesterone, and cortisol.

Hormone assays

Concentrations of FGF21 (product RD291108200R; BioVendor, LLC, Asheville, NC), progesterone (product 4825; Monobind Inc., Lake Forest, CA), and cortisol (product 3625; Monobind Inc., Lake Forest, CA) were determined utilizing commercially available ELISA kits according to manufacturer's recommendations.

Reproductive status

To confirm the reproductive status of the heifers, evaluation of circulating concentrations of progesterone and transrectal ultrasonography were utilized. Heifers were classified as pubertal when circulating concentrations of progesterone exceeded 1 ng/mL. Transrectal ultrasonography was also employed on d 56 to visualize ovarian structures (corpora lutea or albicans) to confirm reproductive status. Heifers were classified as prepubertal if no luteal structures or follicles greater than 10 mm were present on the ovary and circulating concentrations of progesterone did not exceeded 1 ng/mL throughout the experimental period.

Carcass measures

On d 56, ultrasonic measurements of BF were taken between the 12th and 13th ribs. Images were collected using a SonoSite Edge II imaging system equipped with a 10-5 MHz, 15 cm linear array transducer (SonoSite, Inc.; Bothell, WA). Back fat thickness was measured within captured images using integrated software with distance calipers.

Statistical analyses

Circulating concentrations of FGF21, progesterone, and cortisol were evaluated using the MIXED procedure of SAS. The source of variation was d. Day was used as the repeated variable and animal used as the subject. Relationships between reproductive status, ADG, RFI, and BF with concentrations of FGF21, progesterone, and cortisol were determined using the CORR and REG procedures of SAS. Significance was defined as $P \leq 0.05$ and a trend towards significance defined as $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Heifer BW increased linearly from d -7 (273.4 ± 4.2 kg) to d 56 (307.0 ± 5.0 kg) with differences in BW observed between collection dates ($P \leq 0.006$). This increase in BW was expected as heifers were offered a TMR calculated to increase BW throughout the experiment by 0.45 kg per d.

Serum concentrations of FGF21 increased ($P < 0.0001$) from d -7 (411.41 ± 63.45 pg/mL) to d 0 (817.28 ± 63.45 pg/mL) of the feeding period (Figure 1). These results support our hypothesis that circulating concentrations of FGF21 increase when heifers transition from a grazing forage system to a harvested forage system offered in a drylot. The pattern of release of FGF21 in heifers was similar to what Xu et al. (2015) observed in non-lactating and non-gestating dairy cattle in response to changes in dietary energy offered over a period of 35 d. Furthermore, there tended ($P = 0.08$) to be an influence of d on concentration of cortisol with concentrations of cortisol increasing ($P = 0.03$) from d -7 (4.62 ± 0.35 µg/dL) to 28 (5.33 ± 0.35 µg/dL) and then maintained ($P = 1.00$) to d 56 (5.33 ± 0.35 µg/dL). This change in concentration of cortisol overtime is not surprising as animals were exposed to a new social dominance hierarchy as a result of regrouped (Val-Laillet et al., 2008) when entering the GrowSafe system. However, the concentrations of cortisol observed in this experiment were at least 5-fold less than previous reports utilizing restrain in a squeeze chute as a stressor (Alam and Dodson, 1986). Concentrations of FGF21 were not correlated ($P = 0.71$; $y = 0.0002x + 4.945$; $R^2 = 0.001$) with concentrations of cortisol. As a result of these findings, the increased circulating concentrations of FGF21 on d 0 are not likely a result of activation of the stress axis. However, these results contradict previous observations in the FGF21 knockout mouse model illustrating a direct effect of FGF21 on the activation of the stress axis (Patel et al., 2015).

By d 56, concentrations of FGF21 were not different ($P = 0.29$; 497.62 ± 63.45 pg/mL) from concentrations observed on d -7 (Figure 1). We hypothesized that circulating concentrations of FGF21 would decrease

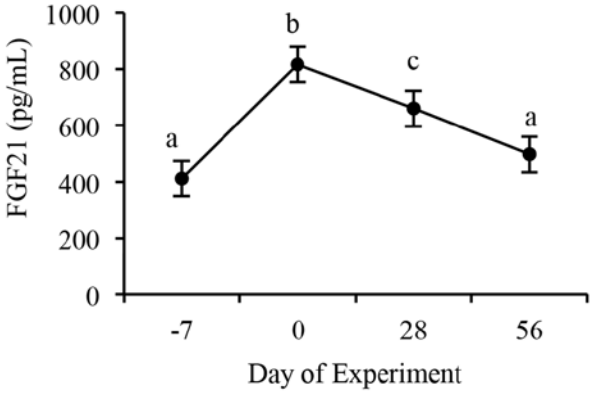

Figure 1. Least squares means (± SEM) serum concentrations of FGF21 in heifers during the experimental period. Means with different letters denote differences between d ($P \leq 0.008$). D $P < 0.0001$

prior to the onset of puberty; however, concentrations did not decrease to concentrations below what was observed on d -7. Furthermore, concentrations of FGF21 were not correlated ($P = 0.11$; $y = -0.0006x + 1.522$; $R^2 = 0.02$) with concentrations of progesterone. Therefore, these data do not support our hypothesis that FGF21 exhibits inhibitory tone that suppresses the onset of puberty in heifers. These results different from Owen et al. (2013) that demonstrated a delay in onset of puberty in transgenic female mice that overexpress FGF21.

Heifers were classified as non-ovulatory (prepubertal) or ovulatory (pubertal) by d 56 based on follicular activity and circulating concentrations of progesterone. As expected, ovulatory heifers (1.48 ± 0.18 ng/mL) had greater ($P = 0.0008$) overall concentrations of progesterone when compared to non-ovulatory heifers (0.35 ± 0.27 ng/mL). Furthermore, no differences ($P = 0.12$) in overall circulating concentrations of FGF21 were evident between groups. However, ovulatory heifers (5.34 ± 0.20 µg/dL) had greater ($P = 0.008$) concentrations of cortisol than non-ovulatory heifers (4.35 ± 0.31 µg/dL). The same has been reported previously by Henricks et al. (1984) when comparing pre- and post-pubertal beef heifers.

We observed a correlation ($y = -0.0002x + 0.5022$; $R^2 = 0.28$) between elevated serum concentrations of FGF21 on d 56 with reduced ($P = 0.002$) ADG. These results support our hypothesis that reduced animal performance is associated with elevated circulating concentrations of FGF21. These results are in agreement with previous data from our laboratory in beef cows (Prezotto et al., 2016). We grouped individual RFI by rank (Low, Mid, or High). When serum concentrations of FGF21 were analyzed by RFI rank, Low RFI heifers (701.77 ± 77.59 pg/mL) had greater ($P = 0.03$) concentrations of FGF21 than High RFI heifers (402.93 ± 105.73 pg/mL; Figure 2). However, neither groups differed ($P \geq 0.15$) from Mid RFI heif-

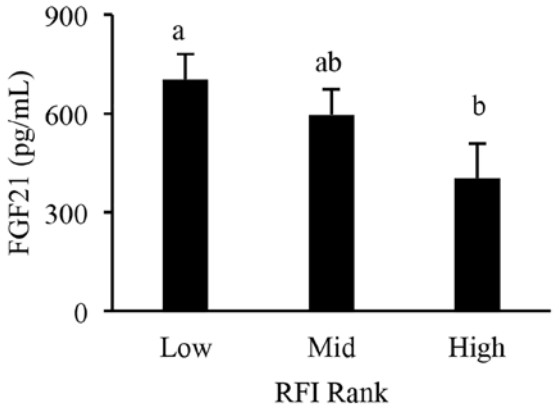

Figure 2. Least squares means (± SEM) serum concentrations of FGF21 in heifers during the experimental period plotted by RFI rank. Means with different letters denote differences between ranks ($P \leq 0.03$). Rank $P = 0.09$.

ers (595.88 ± 77.59 pg/mL; Figure 2). Finally, there was no correlation ($P = 0.31$; $y = -0.00002x + 0.1737$; $R^2 = 0.03$) between serum concentrations of FGF21 on d 56 and BF. These results provide evidence that circulating concentrations of FGF21 may influence some measures of heifer performance.

Implications

The relationship between puberty onset and circulating concentrations of FGF21 was inconsistent with our hypothesis. However, we were able to demonstrate that ADG and RFI rank are correlated with circulating concentration of FGF21. Additional research is needed to determine if concentration of FGF21 affects reproductive performance in heifers.

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L-Lysine and L-Taurine HCL in Nellore young bulls diets without roughage

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ABSTRACT: To evaluate L-Lysine and L-Taurine HCL in ruminant diets without roughage, twenty-four Nellore young bulls were allotted into two groups. The first group was fed without roughage and without antibiotics diet, containing 82 mg/kg ionized amino acids (L-Lysine plus L-taurine HCL) (LT). The second group was fed with diets containing sodium monensin 161 mg/d plus virginiamycin 172.5 mg/d and 15% of the dry matter as sugar cane bagasse in natura (VM). The feedlot time was 91 days. The weight measurements were performed at the beginning of the trial period and monthly, always with water restriction and fasting for 18 hours, even before slaughter. Protozoa ciliates counting and ruminal fermentation variables were analyzed. Measurements in the carcass and the 9-10-11th rib cut physical composition were taken. Samples of *Longissimus dorsi* muscle, aged 0 and 14 days, were analyzed for texture and color. An immunoassay type "Virginiamycin ELISA" from EuroProxima was used to obtain the virginiamycin concentration in *Longissimus dorsi* muscle and liver. A completely randomized design was used. There were higher propionic acid and lactic acid concentration in ruminal liquid, smaller acetic acid to propionate ratio, pH and ammonia nitrogen (NH_3-N) concentration in the rumen from LT group. There were smaller populations of ciliate protozoa in the rumen of LT group. There was no significant effect on weight gain, carcass yield, carcass pH, dressing percentage and temperature immediately after slaughter and 24 hours after slaughter. No significant differences in pelvic and inguinal fats, heart, liver and kidneys weight were observed. No significant differences in commercial cuts weight were observed. There were no differences between the cooking losses. The aged steaks for 14 days from bull fed LT diet were more tender and their colors enhanced and brilliant. We concluded that rumen bacteria that produce lactic acid are more sensitive to virginiamycin plus monensin than L-Lysine plus L-Taurine HCL and that rumen bacteria that produce ammonia nitrogen (NH_3-N) are more sensitive to L-Lysine plus L-Taurine HCL than virginiamycin plus monensin. The L-Lysine and L-Taurine HCL have great benefits to allow ruminants feed diets without roughage and could be convenient in several commercial conditions. In field conditions, the rations without roughage represent advantage, especially regarding its administration to a large amount of animals just once a week or less. In addition, it contributes to improve the desirable characteristics of meat, once it is tenderer and it does not use any antibiotics.

Key words: antibiotics, carcass characteristics, feedlot performance, meat quality
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INTRODUCTION

In order to the positive responses in animal performance, facility of management and economy an increase in the energy density of feedlot diets has been used. However, feeding high-grain diets can predispose animals to metabolic digestive disturbances, such as acidosis (Schwartzkopf-Genswein *et al*., 2003). Therefore, antibiotics have been commonly used in order to achieve greater daily weight gain and control lactic acid producing organisms that result in acidosis. More recently, banning in 2006 of the use of the antibiotics as animal growth promot-

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ers in the European Union has increased demand from producers for feed additive alternative. Some studies have shown the potential of peptide for this purpose.

Several authors observed that nisin provide lower numbers of the Gram-positive bacteria and ruminal anaerobic microorganism (Mantovani and Russell, 2001, Wiedemann et al., 2001, Kišidayová et al., 2003). Monensin and nisin also inhibited amino acid degradation, and nisin was more effective than monensin in controlling the growth of *Clostridium aminophilum* (Callaway et al., 1997). Monensin and nisin decreased acetate to propionate ratio (4.5 to 3.0), total volatile fatty acid production, starch digesting ruminal bacteria (Callaway et al., 1997) and *in vitro* methane production (Sar et al., 2005). Kišidayová et al. (2009) found that the supplementation of nisin provides significant increase in the population of ciliate protozoa, while the supplementation of monensin caused significant decrease in the protozoa population. Monensin had strong antiprotozoic effects in contrast to the stimulatory effects of nisin. Richardson (1976) observed that hydroxymethyl methionine and hydroxymethyl lysine improved N retention, wool growth, daily BW gain, and feed intake compared with control. L-Lysine HCL has been accepted as food preservative of natural origin with a high antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi between pH 3-9. In according to Elwakeel et al. (2012) the neutralization of the alkaline hydroxymethyl lysine products with HCl was considered potential mechanisms for the inhibition of lysine degradation *in vitro*. It is possible that hydroxymethyl lysine itself inhibited microbial growth, enzymes involved in lysine degradation, or both. Elwakeel et al (2012) found low ammonia production reflected an inhibitory effect of the hydroxymethyl lysine products on ruminal microbes such that microbial function was impaired, thereby limiting lysine degradation. Lima et al. (2009) observed decrease of ammonia nitrogen showing potential antimicrobial effect of peptides to protein bypass protect.

With the purpose of gathering more information about L-Lysine and L-Taurine HCL as antibiotics alternative in ruminant diets, the present study was carried out to evaluate the effects of L-Lysine and L-Taurine on ruminal fermentation parameters, protozoa counts, feedlot performance, carcass characteristics, physical carcass composition and meat quality of Nellore bulls fed without roughage diets.

MATERIALS AND METHODS

All animal were cared for and personnel were trained according to the guidelines established by the São Paulo Ethical Committee for Animal Research, Animal ethics approval and permits from the CEUA/IZ/APTA/SAA, were obtained, opinion nº 223-15.

Diet, animal and experimental procedures

Twenty four Nellore bulls were divided in lots. The first lot was fed the control diet containing as sole bulky residue sugarcane in the proportion of 15 % of the total diet and as feed additives 172.5 mg/d virginiamycin plus 161 mg/d of monensin (VM) (n = 12). The second lot was fed the same concentrate without antibiotic and without roughage, containing 82 mg/kg of ionized amino acids mixture (L-Lysine and L-Taurine HCL 99%, pH 9.74) (LT) (n = 12), manufactured by MJ Animal Nutrition®. It was not used a negative control group to avoid metabolic disturbs.

Diet was formulated to match nutrient requirements specified by the NRC (1996) for ADG of 1.4 kg and experimental treatments differed by use of antibiotics or ionized amino acids, both mixed in mineral salt, as described. The ingredients and chemical composition of experimental basal diets are shown in Table 1. Feed ingredients were mixed in a truck-mounted mixer. The concentrate was finely-ground meal to pass through a 3 mm screen. In the conventional treatment, containing roughage and antibiotics, the bulls were fed for ad libitum intake twice daily throughout the study with fresh feed added at 08:00 (40% of total ration) and 15:00 h (60% of total ration). The amount of fresh feed added was adjusted daily based on the amount of feed refusals remaining before the morning feed delivery (at 07:00 h). Refused feed was discarded daily. In the treatment with 100% concentrate, containing L-Lysine and L-Taurine HCL, the ration were delivered to a feed box once a week and the dry matter intake was obtained through amount needed to fill the feed box again. Bulls had free-choice access to water troughs (0.89 by 1.00 by 1.00 m).

At arrival, bulls were dewormed by Doromectina Dectomax (1 mL/50 kg) and vaccinated with Polyvalent Sintoxan T to prevent anthrax, sudden death, tetanus, bovine viral diarrhea virus, and 7-way *Clostridium spp.*. Subsequently were adapted to pens and diets during a 4-wk period, when 85% concentrate diet was fed. The feedlot time was 91 days going from 28 November 2015 to 27 February 2016. The weight measurements were performed at the beginning of adjustment period, at the beginning of the trial period and monthly, always water restriction and fasting for 18 hours, even before slaughter. The initial body weight of the lots fed VM and LT diet were 338 kg and 328 kg and final weight average was 439 kg for both.

TABLE 1. Chemical composition of experimental basal diets

		Diets	
Nutrient	Unit	VM	LT
Dry matter	%	89.33	89.23
Crude Protein	%	18.81	19.74
Crude Fiber	%	12.01	12.98
Ether Extract	%	1.99	2.18
Mineral	%	7.46	5.56
ENN	%	59.74	59.54
FDN	%	26.33	26.88
FDA	%	16.38	17.53
Hemicelulose	%	9.95	9.35
Celulose	%	15.01	15.98
Lignin	%	1.66	1.47
TND	%	69.33	70.13
Nitrogen	g/kg	30.13	31.60
Calcium	g/kg	13.17	7.90
Phosphorus	g/kg	4.73	4.20
Magnesium	g/kg	2.63	2.23
S	g/kg	2.10	1.57
K	g/kg	9.70	9.37
Iron	mg/kg	367	253
Copper	mg/kg	15.69	8.26
Zinc	mg/kg	87.51	50.89
Manganese	mg/kg	55.19	30.19

Carcass characteristics and meat quality

After slaughter, carcasses were skinned by the traditional method of mechanical traction by chain. The liver, kidneys and the fat of inguinal and pelvic area were removed after being weighed.

Measurements of temperature and pH of the carcasses were made at two different times: one hour after slaughter and immediately after the withdrawal of carcasses from the freezer.

Weighing of hot carcasses was performed and subsequently taken to the cold room, where they remained for 24 hours at 2°C temperature. After this period, the carcasses were again weighed, and taken new pH readings. The carcasses were sectioned between the 5th and 6th ribs to the split between front and rear, and later removed the cuts of 9-10-11th left half of the ribs of each animal for subsequent separation and calculation of cut physical composition, according to the methodology Hankins and Howe (1946), consisting in cutting weighing, visual separation with knife muscle, fat and bone, which are weighed separately to achieve the percentage calculations of each component. Also by removing three steaks of approximately 2.5 cm thickness of *Longissimus dorsi* muscles, which were identified and vacuum packed and subjected to 0 and 14 days maturation and after, frozen at -20°C for subsequent analysis of texture and color objectively.

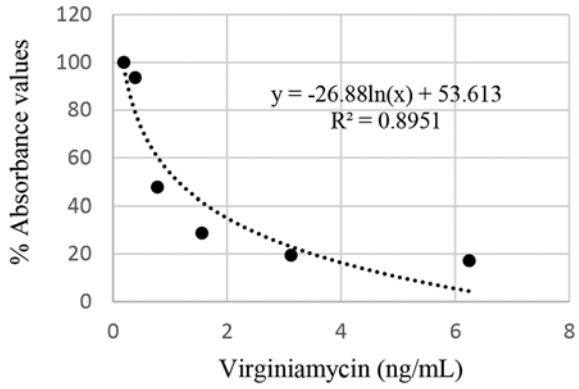

Figure 1. The calibration curve of virginiamycin concentration (ng/mL) and absorbance values. The data points are positive controls from "Virginiamycin ELISA" of EuroProxima.

The analysis of objective texture was carried out according to the methodology proposed by Wheller et al. (1990) and determined by the Warner- Bratzler instrument. The analysis of color was performed only on *Longissimus dorsi*. The space L* a* b*, also called CIELAB, was developed by Cie (1976), the method being used in most all areas which require color measurements. In this space, L* indicates lightness, and a* and b* are the chromaticity coordinates; the axis that runs from -a* to + a* varies from green to red, and -b* to + b* ranges from blue to yellow; the more "close" to the extremities, greater color saturation.

An immunoassay type "Virginimycin ELISA" from EuroProxima (Figure 1) was used to obtain the virginiamicyn concentration in the liver and *L. dorsi*.

The weighing of the half-carcasses was performed with a hanging scale. The *Longissimus* muscle area (LMA) readings were performed with the count cm^2 plastic grid method. Measurements of subcutaneous fat thickness (FT) were performed with use of caliper rule, and the measure taken in the proximal third of the upper portion of the curvature of the *Longissimus dorsi*. The temperature and pH measurements were made using a portable digital measuring temperature and pH at which a metal probe for the temperature reading and punching glass electrode for pH was coupled.

Ruminal fermentation parameters

Ruminal fluid samples were collected on the last day of feedlot period directly through the ruminal after water restriction and fasting for 18 hours. Approximately 500 mL of rumen fluid was collected, at each animal (n=12). Immediately after the collection, 100 mL of rumen fluid was used for pH determination with a portable digital pH meter (model HI8424; HANNA Instruments Ltd., Leighton Buzzard, UK), calibrated with solutions of pH 4.0 and 7.0. For short-chain fatty acids (SCFA)

analyses, a fraction of approximately 100 mL of ruminal fluid was centrifuged at 2000 × g for 20 min and 2 mL of the supernatant was added to 0.4 mL of formic acid and frozen at −20°C for further analyses, according to Erwin et al. (1961). Short-chain fatty acids were measured by gas chromatography (model Focus GC; Thermo Scientific, West Palm Beach, FL, USA) with an automatic injector of samples, equipped with a glass column of 2 m of length and 1/5" of diameter packed with 80/120 Carbopack B-DA/4% (Sigma-Aldrich, St. Louis, MO, USA) and a flame ionization detector maintained at 270°C. The carrier gas was high purity H_2 maintained in flux of 30 mL/min. Lactic acid concentration was measured by a colorimetric technique, according to Pryce (1969). In order to determine ammonia nitrogen (NH_3-N) concentration, 2 mL of the supernatant was added to 1 mL of 1 N of sulphuric acid (H_2SO_4) solution and the centrifuge tubes were immediately frozen at −20°C until the colorimetric analyses, according to the method described by Kulasek (1972) and adapted by Foldager (1977).

Protozoa counts

Rumen content for protozoa counts was collected by scanning the ruminal floor and fixed in 50% formalin (1:1) for microscopic counts, as described by Dehority (1993). Rumen fluid (1 mL) was mixed with 9 mL of methyl green:formaldehyde (38% wt/wt) solution. Entodiniomorphs (Entodinium, Diplodinium and Epidinium) and Holotrichs (Isotricha) were identified and counted using a Neubauer Improved Bright-Line counting chamber (Hausser Scientific Partnership, Horsham, PA, USA).

Statistical analysis

The data were analyzed as completely randomized design using PROC GLM (SAS Institute 2008) with Tukey's mean separation test. A paired t-test was used to determine whether there were significant differences between the treatments. The null hypothesis is that the difference in the mean values is zero, (H0:mA-mB=0). The significance level was declared at $P < 0.05$ unless otherwise noted. Trends for significance were declared at $P = 0.05$ to 0.10.

RESULTS AND DISCUSSION

There was no significant effect on weight gain, carcass yield, carcass pH, dressing percentage and temperature immediately after slaughter and 24 hours after slaughter ($P > 0.05$, Table 2). No significant differences in pelvic and inguinal fats, heart, liver and kidneys weight were observed ($P > 0.05$, Table 2). The dry matter intake observed for lots fed VM and LT diet were 2.59 and 2.31 % of body weight, respectively. In according to body weight gain shown in Table 2, the feed conversion were 8.68 and 6.78 kg of dry matter intake per 1 kg of live weight gain, respectively. Considering that LT group gained plus 120 g/d, despite no significant difference, it was more efficient compared to the VM group. Therefore, depending on the prices of meat, concentrate and roughage, the treatments could result in different economic return.

TABLE 2. Feedlot performance, carcass traits, physical composition of 9-10-11th ribs cut, pH and temperature of carcass and carcass weight of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or L-Lysine plus L-Taurine (LT)

	Diets			
	VM	LT	SEM	*P*-value
Live weight mean, kg	1.202	1.323	0.218	0.244
Carcass weight 0 h, kg	247	250	28.40	0.805
Carcass weight 24 h, kg	244	246	27.83	0.833
Dressing percentage, %	55.68	56.61	2.062	0.280
LMA, cm^2	82.33	84.00	13.44	0.725
FT, mm	5.50	4.83	1.87	0.722
pH in 0 h	6.99	6.99	0.160	0.990
pH in 24h	5.92	5.72	0.307	0.128
Temperature in 0 h, C°	38.16	38.25	0.850	0.812
Temperature in 24 h, C°	5.83	6.25	0.795	0.212
Renal and pelvic fat, kg	2.90	2.43	0.961	0.250
Heart, kg	1.36	1.42	0.309	0.639
Liver, kg	5.39	5.86	0.814	0.172
Kidney, kg	0.85	0.87	0.105	0.668

FT = subcutaneous fat thickness; LMA *Longissimus muscle area.*

There was no significance difference detected in pH values but in the lot fed with LT, the 24 h pH value was between 5.4 and 5.8 that is the normal range according to Roça (1997), while the meat pH values from the lot fed VM 24h after slaughter was 5.9. Meet with elevated pH values (pH>5.8) are associated with DFD (dark, firm and dry) type meet.

No significant differences in commercial cuts weight were observed ($P > 0.05$, Table 3).

There were higher values of yellow color intensity or "b" and higher values red color intensity or "a" in maturing time 14 days of meat from LT group (Table 4). Under normal storage conditions the color is the main attraction of food, showing the amount and chemical state of myoglobin, its most important pigment. The color of fresh meat is determined by the ratio and the distribution of two myoglobin: the ox-myoglobin and metmyoglobin, with red ox-myoglobin, after exposure mus-

TABLE 3. Average yield (kg) of commercial cuts of Nelore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or L-Lysine plus L-Taurine (LT)

	Diets			
Trait	VM	LT	SEM	*P*-value
Hind-quarter (kg)	56.10	57.45	6.51	0.619
Hell (kg)	3.46	3.73	0.52	0.229
Bone (kg)	11.20	11.41	1.17	0.668
Strip loin (kg)	14.68	14.50	1.73	0.798
Tenderloin (kg)	1.85	1.99	0.21	0.114
Neck steak (kg)	1.04	1.04	0.23	1.000
Top sirloin cap (kg)	1.47	1.39	0.20	0.321
Rump steak (kg)	4.69	4.66	0.62	0.922
Knuckle (kg)	4.80	4.78	0.65	0.963
Eyeround (kg)	2.45	2.45	0.34	1.000
Outside flat (kg)	4.96	5.17	0.88	0.570
Topside (kg)	8.44	8.63	1.13	0.683
Parings (kg)	2.05	2.00	0.354	0.691
Fat (kg)	2.90	2.66	0.589	0.342
Spare ribs (kg)	17.09	16.22	1.95	0.289
Forequarter (kg)	50.08	51.19	5.80	0.644
Shank (kg)	2.93	3.13	0.32	0.145
Bone (kg)	9.23	10.06	1.00	0.054
Shoulder clod (kg)	11.78	12.01	1.70	0.741
Chuck (kg)	14.09	14.61	2.45	0.605
Breast rib (kg)	4.71	4.90	0.88	0.616
Hump steak (kg)	3.56	3.33	0.815	0.490
Parings (kg)	0.73	0.63	0.280	0.372
Fat (kg)	3.14	2.81	0.691	0.262

TABLE 4. Means of color parameters of L. dorsi muscle and means of Warner-Bratzler shear force (kg) of L. dorsi muscle, at the different time of maturation of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or L-Lysine plus L-Taurine (LT)

	Diets			
	VM	LT	SEM	*P*-value
Time of maturation (0 days)				
Luminosity	32.07	34.21	4.154	0.221
Red color intensity	12.21	13.08	1.339	0.126
Yellow color intensity	12.31	13.58	1.831	**0.103**
Shear force, kg	61.30	62.71	12.84	0.790
Cooking loss, %	24.58	25.97	3.301	0.317
Time of maturation (14 days)				
Luminosity	38.58	40.00	4.138	0.410
Red color intensity	15.37	16.41	1.278	**0.061**
Yellow color intensity	13.96	15.25	1.539	**0.054**
Shear force, kg	36.44	32.14	6.231	**0.106**
Cooking loss, %	25.30	27.24	3.610	0.201

cle oxygen, responsible for familiar freshness of meat (Seideman et al., 1984; Lawrie, 1985). The shear force in maturing time 14 days was not different from that of VM group (36.4 kg), but there was a downward trend in LT group (32.1) ($P = 0.10$, Table 4). Several studies have shown that tenderness is one of the desirable qualities of meat, at the consumer's point of view, compared with other quality characteristics (Wellington and Stouffer, 1959; Felício, 1993). This way this effect could result in a higher economic return for LT group. In modern meat industry, the variation found in the softness has been identified as the main problem in the final quality of the product. This variation in softness is due to deficiencies in routinely produced carcasses with soft meat and also to identify carcasses that will produce tough meat and rank them according to an established pattern.

It was observed the presence of antibiotic residues of virginiamycin in liver (0.85 ng/mL) and *L. dorsi* (0.75 ng/mL). These levels were quite bellow the Maximum Residue Limits (MRLs) from Codex Alimentarius Commission (10 ng/mL). However, the presence of antibiotics in beef meat is associated with several adverse public health effects including hypersensitivity, tissue damage, gastrointestinal disturbance and bacterial resistant strain (Lee et al., 2001). In view of this, monitoring of residues of these feed additives in animal products meant for human consumption is highly desirable.

There were smaller values of ammonia nitrogen (NH_3-N) concentration in ruminal liquid from LT group ($P < 0.05$, Table 5). In according to Elwakeel et al. (2012) the low ammonia production from hydroxymethyl lysine reflected an inhibitory effect of the hydroxymethyl lysine products on ruminal microbes such that microbial function was impaired, thereby limiting lysine degradation. Even VM group, fed diets containing sugar cane bagasse *in natura* that is quite poor in protein (1.3% of CP), the L-Lysine plus L-Taurine HCL were more effective than virginiamycin plus monensin in controlling the ammonia nitrogen (NH_3-N) production, probably due antimicrobial activity against Gram-positive bacteria. This result reflected an inhibitory effect of the LT on growth of ruminal microbes such that of *Clostridium aminophilum*, an obligate amino acid-fermenting ruminal bacterium that can tolerate low concentrations of monensin. Richardson (1976) observed that hydroxymethyl methionine and hydroxymethyl lysine improved N retention.

There were higher propionic acid concentration in ruminal liquid from LT group ($P < 0.05$, Table 5). The absence of roughage in the diet does not decreased acetic acid, but it decreased the acetic acid to propionate ratio and pH in the rumen ($P < 0.05$, Table 5). However, the fall of pH was not enough to predispose animals to acidosis (6.68 x 6.29). This

TABLE 5. Ruminal pH, VFA, lactic acid and ammonia-N concentration in Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or L-Lysine plus L-Taurine (LT)

	Diets			
	VM	LT	SEM	*P*-value
Ammonia N, mg/dL	26.98	12.37	3.63	**0.007**
pH	6.68	6.29	0.44	**0.043**
Acetic, mM	54.20	55.90	8.69	0.823
Propionic, mM	16.06	50.94	6.88	**0.003**
Butyric, mM	8.51	11.22	4.95	0.540
Isobutyric, mM	2.43	3.93	1.31	0.236
Valeric, mM	1.26	5.76	1.47	0.203
Isovaleric, mM	3.93	1.99	0.53	**0.011**
Total VFA, mM	86.4	129.7	16.83	**0.034**
Acetic:Propionic	3.46	1.11	0.43	**0.002**
Lactic acid, mM	0.21	0.30	0.047	**0.099**

TABLE 6. Total and relative counts of protozoa ciliate in the rumen of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or L-Lysine plus L-Taurine (LT)

	Diets			
	VM	LT	SEM	*P*-value
Total counts x10^3/mL				
Entodinium	61.49	41.76	58.2	0.060
Diplodinium	35.93	41.45	48.9	0.628
Epidinium	25.02	11.70	24.1	0.023
Isotricha	19.36	14.42	19.8	0.198
Relative counts, %				
Entodinium	68.11	32.71	37.55	0.053
Diplodinium	14.33	41.38	31.32	0.074
Epidinium	13.07	0.27	17.05	0.117
Isotricha	4.50	25.62	28.99	0.127

result reflected higher growth of ruminal microbes starch digesters instead fiber digesters. Starch in cereal grains is readily fermented by rumen microorganisms and starch-digesting bacteria produce significant amounts of propionate. It is well known that, as a consequence of starch fermentation, both the acetate to propionate ratio and the pH in the rumen are decreased (Russell, 1998). Beside this, it can assumed that some starch-digesting bacteria produce lactate. There were higher lactic acid concentration in ruminal liquid from LT group ($P < 0.05$, Table 5). These results suggested that rumen bacteria that produce lactic acid are more sensitive to virginiamycin plus monensin than L-Lysine plus L-Taurine HCL. Indeed, *Streptococcus bovis*, one of major starch-digesting bacteria in the rumen, release a great deal of lactate into the rumen (Asanuma and Hino, 2000).

There were smaller populations of ciliate protozoa in the rumen of LT group ($P < 0.05$, Table 6). This effect disagree with Kišidayová et al. (2009) who found that the supplementation of nisin provide significant increase in the population of ciliate protozoa, while the supplementation of monensin caused significant decrease in the protozoa population. It is known that monensin has strong antiprotozoic effects, but in this study this apparently controversial effect was attributed the roughage absence in the diet containing amino acids. We can´t attribute antiprotozoic effects for amino acids.

We concluded that rumen bacteria that produce lactic acid are more sensitive to virginiamycin plus monensin than L-Lysine plus L-Taurine HCL and that rumen bacteria that produce ammonia nitrogen (NH_3-N) are more sensitive to L-Lysine plus L-Taurine HCL than virginiamycin plus monensin, and both feed additives are efficient to avoid acidosis.

IMPLICATIONS

The L-Lysine and L-Taurine HCL have great benefits to allow ruminants feed diets without roughage and could be convenient in several commercial conditions. In field conditions, the rations without roughage represent advantage, especially regarding its administration to a large amount of animals just once a week or less. In addition, it contributes to improve the desirable characteristics of meat, once it is tenderer and it does not use any antibiotics.

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Effects of IgY antibodies on Streptococcus equinus strain JB1 as an antibiotic alternative for improving feedlot performance, ruminal fermentation patterns, and ciliate protozoa counts

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ABSTRACT: Twenty-four Nellore young bulls fed with high-grain diet containing bulky residue sugarcane (15% dry matter) were divided into two groups to evaluate the effects of an avian-derived polyclonal antibody preparation as an antibiotic alternative on ruminant-specific bacteria *Streptococcus equinus* JB1 (IgY-JB1) by assessing feedlot performance, ruminal fermentation patterns, and ruminal counts of ciliate protozoa. The treatments were 172.5 mg/d virginiamycin plus 161 mg/d monensin (VM) or 232 mg/d IgY (IgY-JB1). The weight gain was slightly lower in the VM group (1.285 kg/d) than in the IgY group (1.690 kg/d) (P = 0.077). There were no differences in ruminal pH, molar proportion of acetate and butyrate or lactic acid and NH_3-N concentrations. There was a downward trend in the acetic acid to propionate ratio in the VM group (3.69 mmol/L) compared to that of the IgY-JB1 group (4.33 mmol/L) (P = 0.10). *Isotricha* protozoan counts doubled in the batches fed with IgY-JB1 from 12×10^3/mL to 25×10^3/mL (P = 0.040). The total concentration of lactic acid showed that IgY-JB1 was as effective as antibiotics with respect to the *Streptococcus equinus* control. Considering that the feed conversion rates were 7.65 and 5.95 kg of dry matter intake per 1 kg of live weight gain for lots fed with VM and IgY diets, respectively, and that the IgY group animals gained 405 g/d, IgY-JB1 treatment is certainly more efficient than antibiotic treatment. We conclude that the IgY-JB1 diet could be a convenient method for avoiding antibiotics, contributing to the production of antibiotic-free safe food.

Key words: antibiotics, carcass characteristics, feedlot performance, meat quality
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INTRODUCTION

Livestock production is the most significant activity of Brazilian agribusiness. The country contains the second largest herd of cattle (205 million heads) and is the second largest producer of beef worldwide (IBGE, 2010). The intensive raising of animals has increased the use of veterinary drugs and growth-promoting substances to maximize food production efficiency via the control of the ruminal microbiota.

Monensin and virginiamycin are used commonly to eliminate Gram-positive and protozoan microorganisms associated with undesirable processes in the rumen of animals reared on pasture or in confinement. However, the use of antibiotics in animal feed might result in the selection of resistant microorganisms among the commensal bacteria and transient pathogens of the animal's gastrointestinal tract (Salyers and Whitt, 2005). This occurrence is associated with incorrect therapeutic doses or incomplete treatment and may represent potential public health problems (Russell and Houlihan, 2003). Certain bacterial strains isolated from cattle are resistant to multiple antibiotics and could be sources of potential human health hazard (Evans et al., 2005). Therefore, the European Union has banned the use of antibiotics as food additives since 2006.

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However, since the use of antibiotics is important for producing food of animal origin, efforts have been directed towards finding alternatives that can provide the same benefits as antibiotics along with food safety. Use of antibiotics alleviate metabolic problems due to lactic acid production, enhance animal performance, and increase production costs, albeit by compromising the productivity and competitiveness of livestock production in the country.

The bacterium *Streptococcus equinus* is responsible for metabolic problems (Schwartzkopf-Genswein et al., 2003) caused by fermentation of non-structural carbohydrates, mainly starch and sugars, and production of lactic acid. This bacterium dominates the intestinal flora when animals consume soluble carbohydrate-rich diet, resulting in low ruminal pH, metabolic disorders that cause acidosis, and poor animal performance. Several studies have demonstrated the potential of polyclonal antibodies in inhibiting the growth of unwanted ruminal bacteria. The efficacies of polyclonal antibodies in liquid form containing IgY against *Streptococcus bovis* (ATCC 9809), *Fusobacterium necrophorum* (ATCC 27852) and Escherichia coli (O157: H7) or *Clostridium aminophilum* (ATCC 43895) and *Peptostreptococcus anaerobius* (ATCC 49031) (Camas Inc., Minnesota USA) were compared to that of monensin by several researchers. Blanch et al. (2009) and Marino et al. (2011) observed higher ruminal pH and lower incidences of acidosis. Otero (2008) used antibodies in liquid form in the diet of crossbred Holstein × *Bos indicus* cattle, and observed an increase in the potential degradability of neutral detergent fiber NDF and an increase in the number of *Isotricha* protozoa. Pacheco et al. (2012) observed an increase in dry matter intake; however, they did not observe any differences in bovine blood parameters. Pacheco et al. (2012) reported reduction in rumenites, while Rodrigues et al. (2013) observed no effect. Dilorenzo et al. (2008) used antibodies in the liquid form (2.5 mL/d) and observed higher daily weight gain, better feed efficiency, warm carcass weight gain, increase in subcutaneous fat thickness, and better carcass classification, whereas Pacheco et al. (2012) and Millen et al. (2015) found no difference using antibodies in liquid form or as spray-dried powders, respectively.

The results pertaining to the use of polyclonal antibodies are contradictory. The administration of the liquid form of the antibody to large numbers of animals is impractical. In addition, Bastos et al. (2012) did not observe any effect on the molar concentrations of acetic acid, propionic acid, and butyric acid, total fatty acid concentration, acetic acid: propionic acid ratio, ruminal pH, levels of ammoniacal nitrogen and lactate, and protozoan population using diets containing 73% concentrate (based on dry matter) and spray-dried powders of the Camas Inc. polyclonal antibodies with increasing doses of 0, 1.5, 3.0, and 4.5 g/d. These conflicting results are probably due to differences in the strains and the drying process, and high variation in animal, diet, location, production system (in confinement or pasture), and physiological phase of the animal.

In view of the increasing consumer demand for healthy and natural food, new feed additives that may improve the rumen environment by positive manipulation of fermentation are required. In this study, the effects of a lyophilized avian-derived polyclonal antibody preparation on a specific ruminal bacteria, *Streptococcus equinus* JB1 (IgY-JB1), were examined in terms of ruminal fermentation patterns and ciliate protozoa counts as an antibiotic alternative.

MATERIALS AND METHODS

Research on animals was conducted according to the guidelines of the institutional committee on animal use (protocol number 223-15). Twenty-four young Nellore bulls were divided into two groups. The first lot was fed the diet containing bulky residue sugarcane *in natura* at 15% of the total dry matter supplemented with 161 mg/d monensin plus 172.5 mg/d virginiamycin (n = 12) (VM). The second lot was fed with the same diet; however, 8.5 g of lyophilized yolk containing 232 mg/d IgY was used (IgY) (n = 12) as an antibiotic substitute; both the antibiotics and the antibody were provided via a feed-mixed supplement. The study was designed to evaluate the effects of replacing VM with IgY-JB1 in high-grain diets; accordingly, a negative control group was not used.

Polyclonal antibody preparation

Eighty 25-weeks-old white Leghorn hens were immunized with a vaccine containing *Streptococcus equinus* strain JB1 isolated from bovine rumen fluid (a kind gift from Dr. Hilário Mantovani, Universidade Federal de Viçosa, MG, Brazil). The stock culture was thawed and grown in blood plates for 24–48 hours at 37°C under semi-anaerobic conditions. Colonies were collected by scratching and aseptically transferring to a broth tube with 3 mL saline solution. Turbidity was checked and compared to the McFarland standards to obtain a final density of 5×10^9 cells/mL. The saline solution containing antigens was mixed (50:50) with adjuvants (35 mL PBS plus 175 mg $Al(OH)_3$). For the adsorption of antigens, the adjuvant was added slowly and gradually, followed by 4 hours of constant agitation. The mixture was heated at 60°C for 40 minutes (as required) for inactivation. The vaccine was transferred

to sterile serum bottles, capped, and stored at 4°C until further use (up to 2 weeks). Five hundred microliters of the mixture were deeply inoculated in the pectoral muscles of 80 hens. Eggs were collected daily 5–30 days after the immunization, broken, and the shell, yolk, and egg white were separated. Then, the vitelline membrane was disrupted and the yolk was subjected to lyophilization and delipidation according to Akita and Nakai (1993). The lyophilized yolks were used as feed additive (8.5 g/d). After delipidation, samples of the yolk were analyzed for determining the concentration of IgY using the chicken IgY enzyme-linked immunosorbent assay (ELISA) kit (IRKTAH1109; Innovative Research Inc., Novi, MI, USA) and to monitor activity over time after the initial immunization.

Diet and animal experimental procedures

Diet was formulated to match the nutrient requirements specified by the NRC (1996) for an average daily gain of 1.4 kg, and experimental treatments differed by the use of antibiotics or antibodies, as described previously. The ingredients and chemical composition of basal diets are shown in Table 1. Feed ingredients were mixed in a truck-mounted mixer. The bulls were fed ad libitum twice daily throughout the study with fresh feed added at 08:00 hours (40% of the total ration) and 15:00 hours (60% of the total ration). The ration was delivered to a feed bunk (5 m, with 1.25 m per bull for each pen). The amount of fresh feed added was adjusted daily based on the amount of feed refused before the morning feed delivery (at 07:00 h). Refused feed was discarded daily. The bulls had free-choice access to water troughs (0.89 × 1.00 × 1.00 m).

At arrival, the bulls were dewormed with Doromectin Dectomax (1 mL/50 kg) and vaccinated with Polyvalent Sintoxan T to prevent anthrax, sudden death, tetanus, bovine viral diarrhea virus, and seven clostridial diseases. Subsequently, the animals were adapted to pens during a 4-week period on an 85% concentrated diet. The average initial body weights of the batches fed with VM and IgY-JB1 diets were 404 kg and 375 kg and the average final weights were 458 kg and 446 kg, respectively.

Carcass characteristics and meat quality

After slaughter, the carcasses were skinned by the traditional method of mechanical traction by chain. The liver, kidneys, and fat of the inguinal and pelvic areas were removed and weighed.

The temperature and pH of carcasses were measured twice: 1 hour after slaughter and immediately after the withdrawal of the carcasses from the freezer.

TABLE 1. Ingredient and chemical composition of experiment basal diets

Ingredients (g/100 g DM)	
Maize	57.42
Soybean meal (44% CP)	17.00
Cottonseed	7.15
Mineral mixture[1]	1.70
Sodium chloride	0.85
Limestone	0.88
Bulky residue sugarcane	15.00
Chemical compositions	
Dry matter	90.54
Crude Protein (% DM)	16.69
Crude Fiber (% DM)	16.90
Ether Extract (% DM)	1.99
Mineral Matter (% DM)	7.60
NFC (% DM)	56.84
NDF (% DM)	35.83
ADF (% DM)	23.68
Cellulose (% DM)	19.62
Hemicellulose (% DM)	12.18
Lignin	2.74
TDN (% DM)	67.65
Calcium (g/kg)	11.79
Phosphorus (g/kg)	4.71

[1]Each kilogram contains VA ≥ 120 000 IU; VD ≥ 10 000 IU; VE ≥ 300 IU; Fe ≥ 40 mg; Cu ≥ 450 mg; S ≥ 25 g; Cr ≥ 16 mg; Mg ≥ 28 g; I ≥ 30 mg; Na ≥ 48 g; K ≥ 30 g; Zn ≥ 2000 mg; Mn ≥ 850 mg; Se ≥ 20 mg; I ≥ 30 mg and Co ≥ 100 mg. NFC = Non-fibrous carbohydrates.

The warm carcasses were weighed and subsequently taken to a cold room, where they remained for 24 hours at 2°C. After this period, the carcasses were again weighed and new pH readings were obtained. The carcasses were sectioned between the 5th and 6th ribs to separate the front and the rear, and the left-half 9-10-11th rib cuts were later removed from each animal. The physical composition of cuts was estimated according to the methodology of Hankins and Howe (1946), which consisted of cutting with a knife, weighing, and visual separation of muscle, fat, and bone, and separate weighing of parts to calculate the percentage of each component. Additionally, three steaks of approximately 2.5 cm thickness of *Longissimus dorsi* muscles were vacuum-packed and subjected to 0 and 14 days of maturation, after which they were frozen at -20°C for subsequent objective analyses of texture and color.

The objective analysis of texture was performed according to the methodology proposed by Wheeler et al. (1990) using a Warner–Bratzler instrument. The analysis of color was performed only on the *Longissimus dorsi*. The color space L* a* b*, also called CIELAB, was developed by CIE (1976), and is typically used for color measurements. In this space,

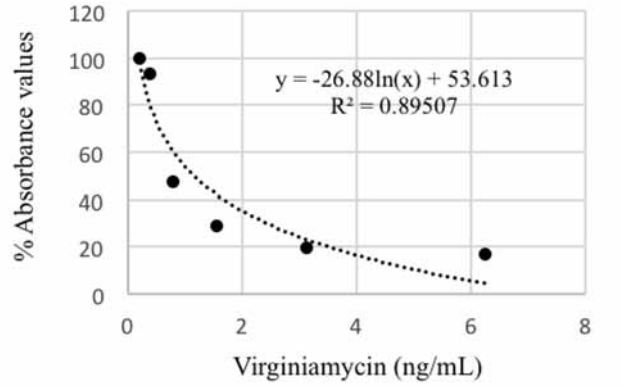

Figure 1. The calibration curve of virginiamycin concentration (ng/mL) and absorbance values. The data points are positive controls from "Virginiamycin ELISA" of EuroProxima.

L* indicates lightness, and a* and b* are the chromaticity coordinates. The axis that runs from -a* to +a* varies from green to red, and -b* to +b* varies from blue to yellow. Values closer to the extremities indicate greater color saturation.

The virginiamycin ELISA kit from EuroProxima (Arnhem, Netherlands; Figure 1) was used to obtain the virginiamycin concentration in the liver and *Longissimus dorsi*.

The half-carcasses were weighed using a hanging scale. The *Longissimus dorsi* muscle area (LMA) was estimated using the counting method and a cm^2 plastic grid. Subcutaneous fat thickness was measured using a caliper, and the measurements were obtained at the proximal third of the upper portion of the curvature of the *Longissimus dorsi*. Temperature and pH were measured using a portable digital temperature and a pH measuring device coupled with a metal probe for the temperature readings and a punching glass electrode for pH estimates.

Ruminal fermentation parameters

Ruminal fluid samples were collected on the last day of the feedlot period directly through the rumen after water restriction and fasting for 18 hours. Approximately 500 mL rumen fluid was collected from each animal. Immediately after the collection, 100 mL rumen fluid was used for pH determination with a portable digital pH meter (model HI8424; HANNA Instruments Ltd., Leighton Buzzard, UK), calibrated with solutions of pH 4.0 and 7.0. For short chain fatty acids (SCFA) analyses, a fraction of approximately 100 mL ruminal fluid was centrifuged at 2,000 × g for 20 min, and 2 mL supernatant was added to 0.4 mL formic acid and frozen at −20°C for further analyses according to Erwin et al. (1961). SCFAs were measured using gas chromatography (model Focus GC; Thermo Scientific, West Palm Beach, FL, USA) with an automatic sample injector equipped with a 2 m-long glass column of 1/5" diameter packed with 80/120 Carbopack B-DA/4% (Sigma-Aldrich, St. Louis, MO, USA) and a flame ionization detector maintained at 270°C. The carrier gas was high-purity H_2 with a constant flow rate of 30 mL/min. The lactic acid concentration was measured using a colorimetric technique, (Pryce, 1969). To determine the ammonia nitrogen (NH_3-N) concentration, 2 mL supernatant was added to 1 mL 1 N sulfuric acid (H_2SO_4) solution and the centrifuge tubes were immediately frozen at −20°C until the colorimetric analyses, according to the method described by Kulasek (1972) and modified by Foldager (1977).

Protozoa counts

The ruminal floor was scanned and the rumen content was collected for estimating the protozoa counts. Then, it was squeezed through cheesecloth into screw-cap glass bottles (100 mL) and fixed in 50% formalin (1:1) for microscopic counts as described by Dehority (1993). Rumen fluid (1 mL) was mixed with 9 mL methyl green: formaldehyde (38% w/w) solution. *Entodiniomorphs* (*Entodinium*, *Diplodinium*, and *Epidinium*) and *holotrichs* (*Isotricha*) were identified and counted using a Neubauer Improved Bright-Line counting chamber (Hausser Scientific Partnership, Horsham, PA, USA).

Statistical analysis

Ruminal fermentation patterns and ruminal counts of ciliate protozoa were analyzed in a completely randomized design using PROC GLM (SAS, 2008) with Tukey's mean separation test. A paired t-test was used to determine significant differences between the treatments. The null hypothesis was that the difference in the mean values was zero, i.e., H0: mA – mB = 0. The significance level was declared at $P < 0.05$, unless otherwise noted. The significance threshold was $P = 0.05–0.10$.

RESULTS

The calibration curve and IgY concentration for lyophilized yolk collected 5, 10, 15, and 30 days after chicken immunization are presented in Figures 2 and 3, respectively. IgY levels at 5, 10, 15, and 30 days after immunizations were 256, 298, 306, and 271 mg/yolk, respectively.

Weight gain was slightly lower in the VM group (1.285 kg/d) than in the IgY group (1.690 kg/d) ($P = 0.07$; Table 2). There were no significant differences in carcass yield, carcass pH, dressing percentage, and temperature immediately after slaughter and 24 hours

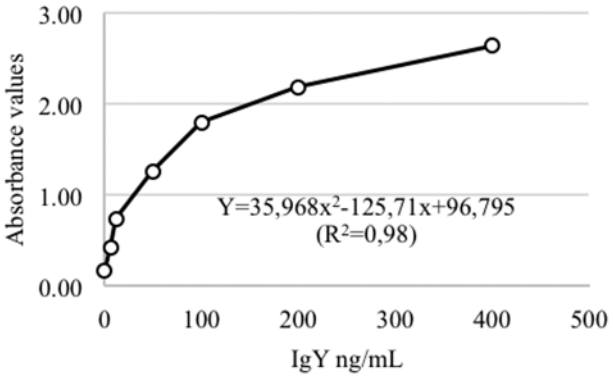

Figure 2. The calibration curve of IgY concentration and absorbance values. The data points are positive controls from "Chicken IgY ELISA KIT" IRKTAH1109, Innovative Research Inc.

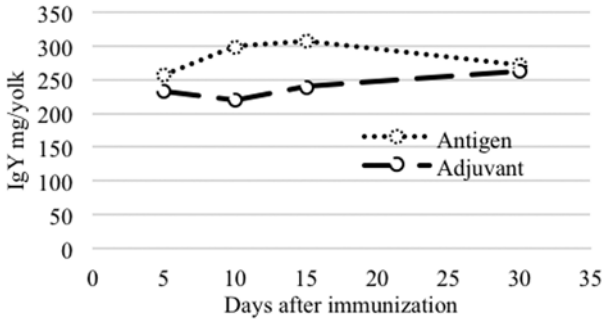

Figure 3. IgY concentration from lyophilized yolk collected in 5, 10, 15 and 30 days after the chicken immunization.

TABLE 3. Average yield (kg) of commercial cuts of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or IgY-JB1 (IgY)

	Diets			
Trait	VM	IgY	SEM	*P*-value
Hind-quarter (kg)	56.10	57.43	6.08	0.668
Hell (kg)	3.46	3.88	0.54	0.148
Bone (kg)	11.20	11.70	1.20	0.426
Strip loin (kg)	14.68	14.51	1.47	0.823
Tenderloin (kg)	1.85	1.90	0.19	0.609
Neck steak (kg)	1.04	0.98	0.19	0.560
Top sirloin cap (kg)	1.47	1.33	0.14	0.075
Rump steak (kg)	4.69	4.71	0.65	0.940
Knuckle (kg)	4.80	5.03	0.65	0.489
Eyeround (kg)	2.45	2.33	0.21	0.262
Outside flat (kg)	4.96	5.33	0.79	0.372
Topside (kg)	8.44	8.55	1.06	0.840
Parings (kg)	2.05	2.01	0.32	0.800
Fat (kg)	2.90	3.15	0.56	0.387
Spare ribs (kg)	17.09	16.40	1.51	0.373
Forequarter (kg)	50.08	51.68	4.50	0.487
Shank (kg)	2.93	3.08	0.30	0.341
Bone (kg)	9.23	10.41	1.03	0.036
Shoulder clod (kg)	11.78	11.93	1.29	0.819
Chuck (kg)	14.09	13.41	1.46	0.369
Breast rib (kg)	4.71	4.63	0.74	0.825
Hump steak (kg)	3.56	3.95	1.28	0.557
Parings (kg)	0.73	1.01	0.29	0.081
Fat (kg)	3.14	3.21	0.62	0.813

TABLE 2. Feedlot performance, carcass traits, physical composition of 9-10-11th ribs cut, pH and temperature of carcass and carcass weight of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or IgY-JB1 (IgY)

	Diets			
	VM	IgY	SEM	*P*-value
Live weight mean, kg	1.285	1.690	0.336	0.077
Carcass weight 0 h, kg	247	248	22.83	0.547
Carcass weight 24 h, kg	244	244	22.49	0.533
Dressing percentage, %	55.17	54.88	1.703	0.772
LMA,[1] cm^2	82.33	77.83	13.61	0.579
FT,[2] mm	5.50	5.00	2.03	0.679
pH in 0 h	7.04	6.95	0.104	0.160
pH in 24h	5.97	6.00	0.234	0.838
Temperature in 0 h, C°	38.33	38.83	0.903	0.360
Temperature in 24 h, C°	6.16	6.16	0.752	1.000
Renal and pelvic fat, kg	3.18	2.71	0.647	0.241
Heart, kg	1.52	1.34	0.354	0.404
Liver, kg	5.49	5.26	0.870	0.657
Kidney, kg	0.88	0.85	0.082	0.623

[1]LMA = Longissimus dorsi muscle area.

[2]FT = subcutaneous fat thickness.

post-slaughter ($P > 0.05$; Table 2). No significant differences in renal and pelvic fat, heart, liver, and kidney weights were observed ($P > 0.05$; Table 2). The dry matter intake observed for lots fed with VM and IgY was 9.84 and 10.08 kg/d or 2.14% and 2.25% of the body weights, respectively. There was no significant difference in carcass pH between groups.

No significant differences in weights of commercial cuts were observed between groups ($P > 0.05$, Table 3). The top sirloin cap weight was slightly lower in the IgY group (1.33 kg/d) than in the VM group (1.47 kg), but this difference was not significant ($P = 0.07$, Table 3). There was higher bone content in the forequarter in the IgY group (Table 3).

There were no significant differences in meat color or in shear force (Table 4). Higher cooking loss (%) was observed in the longissimus dorsi muscle from the IgY group than the VM group (Table 4).

Virginiamycin residues were detected in the liver (0.85 ng/mL) and *Longissimus dorsi* (0.75 ng/mL).

Average ruminal pH, and levels of SCFAs and ammonia-N are shown in Table 5. There were no significant differences in pH and NH_3-N or lactic acid concentrations between the VM group and the IgY-JB1

TABLE 4. Means of color parameters and means of Warner-Bratzler shear force (kg) of L. dorsi muscle, at the different time of maturation of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or IgY-JB1 (IgY).

	Diets			
	VM	IgY	SEM	*P*-value
Time of maturation (0 days)				
Luminosity	37.9	38.1	2.25	0.918
Red color intensity	11.3	11.4	1.77	0.893
Yellow color intensity	10.1	9.7	2.51	0.770
Shear force, kg	62.5	72.7	12.54	0.189
Cooking loss, %	23.5	27.7	3.32	0.051
Cooking loss, g	53.6	56.0	7.02	0.567
Time of maturation (14 days)				
Luminosity	44.8	44.5	3.36	0.858
Red color intensity	16.8	17.1	0.63	0.401
Yellow color intensity	16.1	16.2	1.06	0.941
Shear force, kg	36.7	34.1	4.62	0.368
Cooking loss, %	28.4	28.1	3.07	0.899
Cooking loss, g	56.5	52.1	9.59	0.443

TABLE 5. Ruminal pH, VFA, lactic acid and ammonia-N concentration in Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or IgY-JB1 (IgY)

	Diets			
	VM	IgY	SEM	*P*-value
Ammonia N, mg/dL	21.50	21.37	7.35	0.872
pH	6.68	6.76	0.46	0.736
Acetic, mM	45.76	51.90	16.03	0.678
Propionic, mM	13.22	12.30	5.48	0.822
Butyric, mM	6.24	6.60	0.22	0.898
Isobutyric, mM	2.07	1.99	0.47	0.824
Valeric, mM	0.95	0.72	0.38	0.356
Isovaleric, mM	3.39	2.58	0.73	0.103
Total VFA, mM	71.64	76.71	38.18	0.822
Acetic:Propionic	3.69	4.33	0.65	0.102
Lactic acid, mM	0.19	0.16	0.06	0.475

group. The isovaleric concentration in the IgY-JB1 group (2.58 mmol/L) was not different from that of the VM group (3.39 mmol/L), but there was a downward trend in the IgY-JB1 group (P = 0.10). There was no significant difference in the concentration of total VFA between groups ($P = 0.82$). The acetic acid concentration of the IgY-JB1 group (51.9 mmol/L) was not different from that of the VM group (45.7 mmol/L) and the acetate:propionate ratio did not differ significantly between groups ($P = 0.10$), but was slightly lower in the VM group (3.69) than in the IgY-JB1 group (4.33).

TABLE 6. Total counts of protozoa ciliate in the rumen of Nellore bulls fed a basal diet supplemented with virginiamycin plus sodium monensin (VM) or IgY-JB1 (IgY)

	Diets			
	VM	IgY	SEM	*P*-value
Total counts x10^3/Ml				
Entodinium	66	71	61	0.548
Diplodinium	18	29	28	0.175
Epidinium	21	29	33	0.473
Isotricha	12	25	22	0.040

The counts of ciliate protozoa are shown in Table 6. Supplementation with IgY-JB1 increased *Isotricha* ruminal counts from 12×10^3/mL to 25×10^3/mL ($P < 0.05$).

DISCUSSION

The levels of virginiamycin residue in the liver and *Longissimus dorsi* were below the maximum residue limits established by the Codex Alimentarius Commission (10 ng/mL). However, the presence of antibiotics in beef meat is associated with several adverse public health effects, including hypersensitivity, tissue damage, gastrointestinal disturbance, and emergence of resistant bacterial strains (Lee et al., 2001). Therefore, it is necessary to monitor the amount of antibiotic residues in animal products produced for human consumption.

According to the body weight gain shown in Table 2, the feed conversion rates were 7.65 and 5.95 kg of dry matter intake per 1 kg of live weight gain for lots fed with VM and IgY diets, respectively. Considering that the IgY group gained 405 g/d, the IgY diet was more efficient than the VM diet. Rodrigues et al. (2013) observed that the inclusion of antibodies in diets with 43-29% of roughage improved the feed conversion of Nellore cattle (6.04 vs 6.56). This effect is in agreement with DiLorenzo et al. (2008) who observed higher daily weight gain and better feed efficiency in Angus steers fed with diets containing 19.1% corn silage and supplemented with *Streptococcus bovis* antibodies in liquid form (2.5 mL), However, no effect was observed when the same group used antibodies against *Streptococcus bovis* and *Fusobacterium necrophorum*. In contrast, Pacheco et al. (2012) and Millen et al. (2015) found no difference in weight gain in animals receiving antibodies in liquid form in two daily doses of 5 mL/dose or spray-dried powder (3 g/d), respectively.

In the present study, there was lower carcass yield, although the difference was not significant (54.88 vs 55.17). DiLorenzo et al. (2008) observed a significant reduction in carcass yield upon using antibodies against *Fusobacterium necrophorum* (62.7 vs 62.2)

but not with antibodies against *Streptococcus bovis* (62.5 vs 62.4). Pacheco et al. (2012) also observed a significant reduction in carcass yield when *Bos indicus* was supplemented with 10 mL antibodies compared to animals supplemented with 300 mg of monensin per day (53.4 vs 54.5), which are similar to the results obtained with the Nellore breed in this study.

With the exception of higher bone weight and higher bake loss, there were no differences in carcass characteristics. Rodrigues et al. (2013) and DiLorenzo et al. (2008) also did not observe any differences in the carcass characteristics of Nellore or Angus steers, respectively, upon using antibodies. Probably the effects of improved feed efficiency, higher weight gain, lower carcass yield, and higher bone weight are related to changes in the microbiota and ruminal fermentation pattern. These changes occur due to the differences in the mode of action of antibiotics versus antibodies; the antibiotics act indiscriminately on Gram-positive and protozoan bacteria, whereas the antibodies act specifically against *Streptococcus equinus*. Bone content was higher in the forequarters of the IgY group animals than that in the VM group, probably reflecting continued growth during the feedlot period.

The availability of energy for growth and gain depends on the degradation of dietary nutrients along the digestive tract, which in turn results in the production of organic acids. The proportion of lactic acid (0.19 vs. 0.16 mM, antibiotics vs. antibodies), total short chain fatty acid (71.64 vs. 76.71 mmol, antibiotics vs. antibodies) and acetate:propionate ratio (3.69 vs. 4.33, antibiotics vs. antibodies), demonstrate changes in ruminal fermentation. The antimicrobial effects of the additives used influence the digestibility of NDF and its consumption and flow from the digesta to the intestine. Otero (2008) observed an increase in the potential degradability of sugarcane NDF in animals supplemented with antibodies in liquid form and maintained at 4.4-10^{o}C. Increase in NDF degradability enhances the production of acetic acid, dry matter intake, and passage rate, which reduces the acetate:propionate ratio. It is likely that the VM group had lower numbers of Gram-positive bacteria other than *Streptococcus equinus*, such as *Ruminococcus albus* (Guo et al., 2010), which uses cellulose and hemicellulose to produce acetate (Russell and Rychlik, 2001), whereas IgY-JB1 was more specific than VM and resulted in lower numbers of only *Streptococcus equinus* strain JB1 in the antibody-treated group. Although there was no difference in total SCFA (71.6 and 76.7 for VM and IgY-JB1, respectively), the effect of IgY-JB1 on bacteria was more specific than that of VM.

Otero (2008) demonstrated that supplementation with antibodies in the liquid form reduced the soluble fraction of starch by 45.26% and 45.37% compared to the control group and the monensin-supplemented group, respectively. The intestine is more efficient in utilizing the energy of starch digestion than the rumen since energy is not lost in the production of heat of fermentation in the intestine. According to Orskov (1986), these losses, mainly through the formation of heat and gases, amount to 12-20% of the ingested energy. Owens et al. (1986) concluded that starch digested in the intestine offers 42% more energy than that digested in the rumen. In this study, the food consumption of animals was higher than those of animals supplemented with antibodies (2.25 vs. 2.14), which is in agreement with the results of Millen et al. (2015), and Pacheco et al. (2012). Rodrigues et al. (2013) observed that monensin supplementation reduced dry matter intake compared to antibody supplementation. Thus, the greater consumption and passage rate of starch to the intestine, combined with less ruminal degradation of starch, may have favored the use of energy from this nutrient in the intestine, resulting in greater weight gain. However, the digestion of starch in the intestine may not be without limitations because of the following reasons (Nocek and Tamminga, 1991): the pancreas may not secrete sufficient amounts of amylase, maltase or isomaltase in the time required for efficient digestion; the passage rate may limit complete hydrolysis of starch; the physical structure, insolubility or impermeability of starch granules might limit their accessibility to enzymes. Thus, the use of antibodies might limit the growth of starch-degrading bacteria in the rumen and cause adverse effects. Marino et al. (2011) observed lower digestibility of starch in animals receiving antibodies in liquid form when compared to animals receiving monensin sodium in diets with 70% concentrate.

The lack of effect of polyclonal antibodies on the total production of short chain fatty acids and the molar ratio of acetic, butyric, propionic, lactic, and ammoniacal acids are in agreement with the results of Marino et al. (2011), who observed no effect on these same parameters when comparing supplementation with antibodies in liquid form with sodium monensin supplementation. These results, particularly the similarities in lactic acid concentration in the two groups, show that IgY-JB1 is effective in the control of the lactic acid-producing *Streptococcus equinus* strain JB1. However, the downward trend in the acetate: propionate ratio (4.33 vs. 3.69, IgY-JB1 vs. VM, respectively) suggests that VM results in lower numbers of *Isotricha* protozoa and Gram-positive bacteria, whereas IgY-JB1 is specific for *Streptococcus equinus* strain JB1. Marino et al. (2011) also observed higher acetate: propionate ratio when comparing the effects of polyclonal antibodies in liquid

form with that of monensin sodium (3.24 vs. 2.50). The higher counts of ciliate protozoa probably contributed to the effect on the acetate: propionate ratio as *Isotricha* species produce acetic acid, but not propionic acid. Otero (2008) observed a 93.65% increase in *Isotricha* counts upon using antibodies in liquid form compared to that of the control group, but not in comparison to the group that received monensin sodium.

The absence of the effect of antibodies on the pH of the ruminal liquid in this study are in agreement with the results of Blanch et al. (2009) and Marino et al. (2011). Marino et al. (2011) demonstrated higher ruminal pH values in animals receiving antibodies in liquid form compared to that of the control treatment (without monensin or antibodies); however, in the same experiment, there was no difference between the treatments with monensin or antibodies. The main reason for this could be the absence of a negative control group in this study; a negative control group was not used because the study aim was to evaluate the effects of replacing VM with IgY-JB1 in high-grain diets. Probably, a significant difference between the pH values of ruminal liquid would be observed upon comparison with a control treatment without antibiotics and antibodies. Therefore, these results are different from those of Bastos et al. (2012) who did not detect differences in the rumen pH, SCFA concentration, molar proportions of acetate, propionate, and butyrate, ammonia nitrogen (NH_3-N) and lactate while comparing of a spray-dried product treatment with the control treatment. These differences may occur depending on the form of presentation and method of drying the products (spray-dried or lyophilized).

These results suggest that lyophilization is an excellent method for preserving a wide variety of heat-sensitive materials, and that immunization with the *Streptococcus equinus* JB1 strain was effective for antibody production. Considering that the feed conversion rates were 7.65 and 5.95 kg of dry matter intake per 1 kg of live weight gain for lots fed with VM and IgY diets, respectively, and that the IgY group animals gained 405 g/d, IgY-JB1 treatment is certainly more efficient than antibiotic treatment. We conclude that the IgY-JB1 diet could be a convenient method for avoiding antibiotics, thereby contributing to the production of safer antibiotic-free food.

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Effects of injectable trace-mineral supplementation on the reproductive development of beef bulls

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ABSTRACT: We evaluated effects of two supplemental s.c. trace mineral injections on growth and breeding soundness of bull calves. Weaned bulls (n = 491; initial BW = 314 ± 45 kg, initial age = 203 ± 14 d) of 3 breeds (Charolais, Angus, and Charolais × Angus) and originating from 12 ranches in the Great Plains were blocked by breed type and ranch of origin and assigned randomly to 1 of 2 treatments: 1) s.c. injections of trace mineral (TM) containing 15 mg/mL Cu, 5 mg/mL Se, 10 mg/mL Mn, and 60 mg/mL Zn or 2) s.c. injections of physiological saline (SA). Treatments were administered at weaning (d 0; 1 mL/45 kg BW) and 90 d after weaning (1 mL/68 kg BW). Bulls were stratified by treatment, breed, and ranch of origin and assigned randomly to 1 of 8 pens where they were fed a growing diet *ad libitum* for 225 d. The diet was formulated to promote a 1.5 kg ADG at a DMI of 2.6% BW and to meet or exceed NASEM (2016) requirements for Ca, Co, Cu, I, Mg, Mn, P, K, Se, Na, and Zn. Initial BW and pretreatment plasma samples were collected on d 0. Breeding soundness (BSE) and BW were assessed at 10 and 12 mo of age (d 90 and 150, respectively). Scrotal circumference was measured and semen samples were collected via electro-ejaculation. Sperm-cell motility and morphology were evaluated via light microscopy by a single technician. Body weight, ADG, and scrotal circumference did not differ ($P \geq 0.16$) between the treatment groups at any time point. Proportions of TM- and SA-treated bulls receiving satisfactory BSE scores did not differ ($P = 0.98$) at 10 months of age (50% for both TM and SA). At 12 months of age, the proportion of TM-treated bulls receiving satisfactory BSE scores was numerically larger ($P = 0.43$) than that of SA-treated bulls (TM = 89%, SA = 86%). This change was associated with greater ($P = 0.05$) sperm motility in TM-treated bulls than in SA-treated bulls at 12 months of age; moreover, TM-treated bulls had greater ($P \leq 0.05$) improvement in sperm morphology and sperm motility between 10 and 12 months of age than SA-treated bulls. Among bulls that failed BSE at 10 months of age, more ($P = 0.10$) TM-treated bulls passed BSE at 12 months of age than SA-treated bulls (98 vs. 94%, respectively). Under the conditions of this study, sperm motility and morphology at 12 months of age were improved in bulls treated with injectable TM at 7 and 10 months of age compared to bulls treated with SA.

Key words: breeding soundness, sperm motility, sperm morphology, trace minerals
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INTRODUCTION

The cost associated with developing young bulls to sexual maturity is highly variable. Development costs motivate breeders to minimize the number of animals that are culled due to reproductive failure. Many breeders develop young bulls in a feedlot environment for some length of time after weaning to ensure adequate growth and timely puberty. Bull-development diets fed in confinement are generally formulated to meet or exceed NASEM (2016) recommendations for trace minerals; however, variation in DMI and gut-level antagonisms may limit mineral intake or absorption.

Injectable supplemental trace minerals may be used to bypass gut-level antagonisms and to overcome poor intestinal absorption and variation in trace-mineral intake. Minerals of particular significance to sexual development of bulls include Cu, Mn, Se, and Zn. They have integral roles in either spermatogenesis (Underwood and Somers,

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1969); sperm motility and morphology (Brown and Burk, 1973; Swarup and Sekhon, 1976; Hunter, 1977; Parillo et al., 2014); testicular hypertrophy (Miller and Miller, 1962); tissue repair (Machado et al., 2014); or steroid hormone synthesis (Hurley and Doane, 1989). Due to the relatively high value of breeding bulls compared with non-breeding cattle, small improvements in breeding soundness achieved through investing in injectable trace-mineral supplementation may result in greater net revenue for breeders. Therefore, the objective of our study was to evaluate the performance and breeding soundness of weaned bull calves subject to two supplemental s.c.injections of either trace minerals or physiological saline.

TABLE 1. Composition of the diet fed to weaned bulls

Ingredient composition	% DM
Corn silage	40.3
Modified distillers grains	35.1
Ground prairie hay	22.5
Supplement[1]	2.1
Nutrient composition[2]	DM basis
CP, % DM	14.1
NE_m, Mcal/kg DM	1.57
NE_g, Mcal/kg DM	0.97

[1]Supplement contained ammonium sulfate, limestone, urea, salt, Rumensin 90® (300 mg $head^{-1} \cdot d^{-1}$), and a trace-mineral premix.

[2] Diet was formulated to meet 100% of NASEM (2016) requirements for Ca, Co, Cu, I, Mg, Mn, P, K, Se, Na, and Zn.

MATERIALS AND METHODS

Animal care practices used in our study were reviewed and approved by the Kansas State University Animal Care and Use Committee (protocol no. 3426). Weaned, fall-born bull calves (n = 491; initial BW = 314 ± 45 kg; initial age = 203 ± 14 d) of 3 breeds (Charolais, Angus, and Charolais × Angus) originating from 12 ranches across Kansas, Oklahoma, and Colorado were used in this study. At the initiation of the study on April 21, 2014, bull calves from all ranches were shipped to a feedlot near Randolph, KS. Upon arrival, each bull was identified with a unique visual ear tag and a radio-frequency transponder button (half-duplex RFID, Allflex USA Inc.; Ft Worth, TX). Bulls were weighed and assigned randomly to 1 of 2 treatments: supplemental s.c. trace mineral injections (Multimin® 90; Multimin USA Inc., Fort Collins, CO; **TM**) or subcutaneous injections of physiological saline (**SA**). Both treatment were administered at study initiation (1 mL/45 kg BW) and again 90 d later (1 mL/68 kg BW). Each subcutaneous TM injection provided 15 mg/mL Cu, 10 mg/mL Mn, 5 mg/mL Se, and 60 mg/mL Zn.

Bulls were stratified by treatment, breed, and ranch of origin and randomly assigned to 1 of 8 pens (minimum area = 200 m^2 / calf; bunk space = 0.46 m / bull) and afforded *ad libitum* access to water via concrete tanks. Treatments were represented equally in all pens. A growing diet (Table 1) formulated to promote a 1.5-kg ADG at a DMI of 2.6% of BW was fed for 225 d. Bunks were evaluated each morning at 0630 h, and feed was delivered once daily at 0700 h. Bunks were managed using a slick-bunk management method to minimize feed refusals. If all feed delivered to a pen was consumed, delivery at the next feeding was increased to approximately 102% of the previous delivery. Diet samples were collected from bunks weekly and frozen at -20°C. Samples were composited by weight at the conclusion of the study and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, and ADF (Table 1). Diet NE values were calculated from detergent fiber analyses using equations provided by NASEM (2016).

Bulls were weighed individually on d 0 (study initiation), d 90, and d 150 at 0600 prior to feed delivery. Neither BW nor ADG differed ($P \geq 0.16$) between the treatment groups at any time point. (data not shown). This was expected as all bulls consumed similar amounts of the same diet.

Pretreatment blood samples were taken via caudal vessel puncture on d 0. Serum mineral concentrations were analyzed for Cu, Mn, Se, and Zn concentrations via inductively coupled plasma spectrometry (Varian ICP, Santa Clara, CA). Serum mineral concentrations were not different ($P \geq 0.42$) between treatments (data not shown).

Breeding soundness examinations (**BSE**) were administered on d 90 and 150 at 10 and 12 mo of bull age, respectively. A single veterinarian measured scrotal circumference and collected semen samples using a programmable electro-ejaculator. Semen samples were assessed visually for motility and morphology by a single technician immediately following collection. Bulls with white blood cells in ejaculate were presumed to have vesiculitis and treated using a macrolide antibiotic (Draxxin®; Zoetis Inc., Kalamazoo, MI) under the supervision of a veterinarian. Breeding soundness classifications of satisfactory or unsatisfactory for breeding were assigned as specified by the Society of Theriogenology. Semen samples presenting < 30% motility, < 70% normal morphology, or a scrotal circumference < 30 cm resulted in a failed BSE. Bulls that passed the BSE on d 150 were marketed via live auction on d 225 at a approximately 15 mo of age.

Bulls were monitored daily during our study for symptoms of respiratory disease, interdigital infection, and conjunctivitis. Bulls with clinical signs of illness,

as judged by animal caretakers, were removed from pens and restrained. Bulls with suspect respiratory disease were assigned a clinical-illness score (scale: 1 to 4; 1 = normal, 4 = moribund) and assessed for febrile response. Bulls with a clinical illness score > 1 and a rectal temperature > 40.0°C were treated with therapeutic antibiotics according to label directions. Bulls with signs of conjunctivitis were treated with Micotil 300® (Elanco Animal Health, Eli Lilley & Co., Greenfield, IN), whereas those with acute necrotic infection of the interdigital skin were treated with Nuflor® (Merck Animal Health, Intervet Inc., Madison, NJ).

Results were analyzed as a randomized, incomplete block (PROC MIXED; SAS Inst. Inc., Cary, NC). Bull within treatment and pen was the experimental unit. Class factors included ranch of origin, pen, breed, and treatment. The model statement included terms for the fixed effects of treatment, breed, and treatment × breed. Ranch of origin within pen was treated as a random variable. Continuous variables were analyzed using a mixed model, whereas binomial variables associated with breeding soundness were analyzed using logistic regression. When protected by a significant *F*-test ($P \leq 0.05$), Least Squares treatment means were separated using the method of Least Significant Difference. Means were considered different when $P \leq 0.10$.

RESULTS AND DISCUSSION

Pretreatment serum mineral concentrations were analyzed to evaluate differences between breeds (Table 2). In general, Angus bulls had greater ($P \leq 0.05$) serum Cu, Mn, Mo, and Se than Charolais bulls or Angus × Charolais bulls, whereas Angus bulls had lesser ($P \leq 0.05$) serum Co than Charolais bulls or Angus × Charolais bulls. Serum Zn concentrations of Angus and Angus × Charolais bulls were greater ($P \leq 0.05$) than those of Charolais bulls. Serum Cu differences between breeds have been documented (Ward et al., 1995). Angus cattle required less copper in their diets than Charolais and Simmental cattle (Herd, 1997). Little is known about serum Se differences between breeds (Hohenboken and McClure, 1993).

Notably, serum Mo concentrations reported in table 2 were well above normal for growing bulls. Young bulls fed high levels of Mo lacked libido and exhibited degeneration of seminiferous tubules and testicular interstitial tissue (Thomas and Moss, 1951).

Breeding soundness exams (BSE) were administered on d 90 (**BSE1**) and 150 (**BSE2**) at 10 and 12 mo of bull age, respectively. Proportions of TM- and SA-treated bulls receiving satisfactory BSE1 scores did not differ ($P = 0.98$; 50% for both TM and SA).

TABLE 2. Breed effects on pretreatment blood serum mineral concentrations* of weaned beef bulls

	Breed			
Item	Black Angus	Charolais	Black Angus x Charolais	SE
Co, ppb	0.96[a]	1.09[b]	1.15[b]	0.034
Cu, ppm	0.63[a]	0.57[b]	0.59[b]	0.011
Fe, ppm	158.88	153.30	174.65	18.260
Mn, ppb	2.13[a]	1.81[b]	1.90[b]	0.097
Mo, ppb	47.97[a]	0.0[b]	0.0[b]	18.164
Se, ppb	69.09[a]	65.27[b]	67.48[b]	0.715
Zn, ppb	1.00[a]	0.87[b]	1.11[a]	0.081

*Pretreatment blood serum samples collected on d 0.

[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

The proportion of TM-treated bulls receiving satisfactory BSE2 scores was numerically larger ($P = 0.43$) than that of SA-treated bulls (89 and 86 % for TM and SA, respectively). Of bulls that failed BSE1, 4% more ($P = 0.10$) TM-treated bulls passed the BSE2 than SA-treated bulls. The proportions of bulls that either passed or failed both BSE1 and BSE2 were not different ($P \geq 0.66$) between TM and SA.

Scrotal circumference did not differ ($P \geq 0.16$) across treatments for either BSE1 or BSE2. The proportion of normal sperm cells at BSE1 and BSE2 was not different ($P \geq 0.33$) between treatments; however, the percentage change in normal sperm cells from BSE1 to BSE2 was greater ($P = 0.05$) for TM than SA. Several authors noted that Se and Zn were particularly critical for normal spermatogenesis and midpiece and tail morphology (Underwood and Somers, 1969; Swarup and Sekhon, 1976; Parillo et al., 2014).

Satisfactory sperm motility percentage at both BSE1 and BSE2 was unaffected ($P \geq 0.47$) by treatment; however, the percentage of motile cells was greater ($P = 0.05$) at BSE2 for bulls assigned to TM than SA. In a related effect, TM-treated bulls had a greater ($P = 0.04$) percentage change in sperm motility from BSE1 to BSE2 than SA-treated bulls. Selenium has been identified as an essential mineral for male rodent fertility, with deficiencies resulting in reduced sperm motility and an increased number of abnormal sperm cells (Brown and Burk, 1973). Hunter (1977) reported improved sperm motility when Cu was added to bull diets.

Ejaculate samples were examined via light microscopy for the presence of white blood cells (**WBC**); the presence of WBC constituted automatic BSE failure. The proportion of bulls that failed BSE1 or BSE2 because of the presence of WBC in semen were not different ($P \geq 0.35$) between TM and SA (Table 3).

Bulls were monitored daily for symptoms of conjunctivitis or interdigital infection. The percentage of

TABLE 3. Effects of bolus injections of either a trace mineral solution or physiological saline (1 mL/90 kg BW) on breeding soundness, scrotal circumference, sperm morphology, and sperm motility of weaned beef bulls

	Treatment			
Item	Saline[a]	Trace Mineral[b]	SE	*P*-value
Satisfactory BSE1[c], %	50.3	50.2	3.85	0.97
Satisfactory BSE2[d], %	86.4	88.8	2.18	0.43
Change in BSE (BSE2 – BSE1), %	36.1	38.9	3.94	0.54
Failed BSE1 but passed BSE2, %	93.7	97.7	2.09	0.10
Passed both BSE1 and BSE2, %	47.5	49.4	3.76	0.66
Failed both BSE1 and BSE2, %	11.1	10.0	2.10	0.80
Normal sperm cells at BSE1, % of total	56.9	54.9	2.46	0.46
Normal sperm cells at BSE2, % of total	70.3	73.2	1.42	0.13
Change in normal sperm cells from BSE1 to BSE2, %	13.6	18.7	2.25	0.05
Scrotal circumference at BSE1, cm	34.1	33.7	0.29	0.16
Scrotal circumference at BSE2, cm	36.6	36.5	0.25	0.45
Change in scrotal circumference from BSE1 to BSE2, cm	2.6	2.8	0.14	0.26
Satisfactory scrotal circumference at BSE1, %	93.4	90.7	2.04	0.27
Satisfactory scrotal circumference at BSE2, %	99.6	99.2	0.50	0.58
Satisfactory sperm morphology at BSE1, %	53.2	53.7	3.85	0.91
Satisfactory sperm morphology at BSE2, %	87.7	90.4	2.11	0.33
Sperm motility at BSE1, % motile cells	33.8	33.3	1.02	0.74
Sperm motility at BSE2, % motile cells	40.2	42.2	0.86	0.05
Change in sperm motility from BSE1 to BSE2, %	6.2	8.7	1.12	0.04
Satisfactory sperm motility at BSE1, %	78.8	76.1	2.89	0.47
Satisfactory sperm motility at BSE2, %	91.4	92.8	1.99	0.55
White blood cells in semen at BSE1, %	4.9	3.2	1.32	0.35
White blood cells in semen at BSE2, %	2.1	1.6	0.86	0.71
Treated once for conjunctivitis or interdigital infection[e], %	41.1	43.4	4.37	0.59
Treated twice for conjunctivitis or interdigital infection[e], %	1.3	1.9	8.66	0.94

[a] Bulls injected with physiological saline on d 0 (1 mL/45 kg BW) and d 90 (1 mL/68 kg BW).

[b] Bulls treated with injectable trace-mineral supplement on d 0 (1 mL/45 kg BW) and d 90 (1 mL/68 kg BW).

[c] Initial breeding soundness exam (BSE1) was conducted on d 90 at approximately 10 months of age.

[d] Final breeding soundness exam (BSE2) was conducted on d 150 at approximately 12 months of age.

[e] Duration of the development period was 225 d; treatments may have occurred any time during this period.

bulls treated once or twice for either infection was not affected ($P \geq 0.59$) by treatment.

The bulls used in our study were developed with the intention of marketing them as breeding stock during October 2014. Sale averages and treatment costs were averaged across breed groups. Bulls that passed BSE2 were marketed via live auction and averaged $7,500 / bull. Bulls that failed BSE2, but passed a third BSE 60 d later, were marketed private treaty for an average of $6,000 / bull. Bulls that failed the third BSE were sold as non-breeding culls at a local auction market for an average price of $2,040 / bull.

The treating of weaned bulls with supplemental subcutaneous trace mineral injections at 7 and 10 months of age cost $5.88 / bull. The addition of a $3 / bull chute charge (2x), resulted in a total treatment cost of $11.88 / bull or $1,188 / 100 bulls. Per 100 TM-treated bulls, 89 were eligible for live auction, 6 for private treaty, and 5 for non-breeding culls, creating a gross revenue of $713,700. Per 100 SA-treated bulls, 86 were live-auction eligible, 8 were private-treaty eligible, and 6 were non-breeding culls, resulting in a gross revenue of $705,240. The gross difference between the two treatment groups, minus TM treatment cost, resulted in a net treatment benefit of $7,272 / 100 bulls or $72.72 / bull.

IMPLICATIONS

Under the conditions of our study, the proportion of young bulls meeting minimum passing requirements for breeding soundness was not different between bulls treated with injectable trace minerals or treated with saline; however, sperm motility and morphology scores were improved in bulls treated with trace minerals compared with those treated with saline. Of bulls that failed breeding soundness evaluations at 10 mo of age, 4% more trace-mineral treated bulls

passed breeding soundness evaluations at 12 mo of age than saline-treated bulls.

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The effects of delayed processing on hydration status, health, and performance of newly received feedlot heifers[1]

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ABSTRACT: This study evaluated the effects of delayed processing on calf hydration status, subsequent health and performance. Crossbred heifers (n = 224; BW = 187 ± 3.2 kg), blocked by 2 truckloads (114 and 115 calves per truckload), assigned to 16 pens and two treatments were used in a randomized complete block design. Treatments were initial processing upon arrival (ARV) or a 24 h delayed processing (REST), after 12 h of transportation episode. During the 24 h delay, REST calves were allowed access to wheat hay, a receiving ration, and fresh drinking water. Initial processing included heifers being weighed, ear tagged, dewormed, vaccinated and no metaphylactic antibacterial treatment. On d 1, 2, 3, and 28, individual BW, and rectal temperatures were obtained. Venous blood samples collected on d 0 (ARV only), 1, 2, 3 and 14, and were used for hematocrit measurement as indicator of hydration status. Health was monitored throughout the 56-d experimental period and pen weights were obtained on d 56. Performance and hematocrits were analyzed using MIXED procedures. Morbidity and mortality data were analyzed using GLIMMIX procedures. Receiving protocol (delayed processing) did not affect heifer hematocrits, health, or performance ($P \geq 0.10$). Blood hematocrits was lower ($P < 0.01$) on d 14 than d 1, 2, and 3 and had a tendency for a treatment × day interaction ($P = 0.12$); on d 1, 2, and 14 blood hematocrits were not different, but were greater ($P < 0.05$) for REST than ARV calves on d 3. Total medical treatments tended ($P = 0.14$) to be greater for REST than ARV calves. Calf ADG and G:F tended to be lower ($P \leq 0.13$) for REST than ARV calves from d 1 to 28, and d 1 to 56. In conclusion, delaying initial processing by 24 h after a 12-h transportation episode is not enough time to allow calves to sufficiently rehydrate to observe health and performance benefits. Postponing initial processing delays exposure to many stress factors and may cause dehydration to the same extent as 12 h of transportation.

Key words: dehydration, feedlot, health, heifer, hematocrit
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INTRODUCTION

Bovine respiratory disease (**BRD**), shipping fever, is the most significant cause of morbidity and mortality in newly received feedlot calves. In 2011, shipping fever affected 21.2% of calves (BW < 318 kg) placed on feed (USDA, 2011). Water is often neglected as the most important nutrient to the animal. Calves arriving at the feedlot are subjected to prolonged periods of restricted access to water during marketing, weaning, and transportation. Richeson et al. (2013) reported hematocrit percentages of 36.9 ± 3.7% for calves arriving at the feedlot. Parker et al. (2004) reported hematocrit percentages of 42.5 ± 1.5% for calves deprived from feed and water, and transported for 48 h. Normal bovine hematocrit values range from 25 to 33% (UC Davis College of Veterinary Medicine, 2011; Cornell University College of Veterinary Medicine, 2014), which would imply that the elevated hematocrit percentages reported by Richeson et al. (2013) and Parker et al. (2007) are consistent with dehydration.

Dehydration and stress cause decreased preciliary thickness and movement, and a decrease in the water content of respiratory mucus, both

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compromising the animal's ability to clear inhaled pathogens (Constantinopol et al., 1989; Jones et al., 1989; Caswell, 2013). Once pathogens cross immune barriers, antibody responses provide the ultimate solution to recognize invading pathogens to ensure their destruction (Tizard, 2009) but are suppressed by transportation stress (Kegley et al., 1997). Therefore, we hypothesized that allowing heifers 24 h rest upon arrival, with access to feed and water would allow calves to rehydrate before initial processing the next day. We also hypothesized that the improved hydration status would be evident in blood hematocrit percentages and enhance subsequent immunocompetence and performance of calves. The objective of this study was to evaluate the effect of rest upon arrival on calf hydration status, health, and performance.

MATERIALS AND METHODS

All animal procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. This experiment was conducted at the Clayton Livestock Research Center with 224 crossbred heifers (initial BW = 187 ± 3.2 kg).

Experimental Design and Treatments

The experiment was a randomized complete block design, blocked by 2 truckloads (114 and 115 calves per truckload) with pen as experimental unit. Upon arrival, calves within a block were randomly assigned to 8 pens (14 calves per pen) and pens of calves were randomly assigned to two treatments (8 replicated pens per treatment). The two treatments consisted of receiving protocols where calves shipped from south-eastern Texas (12 h on truck; 1,159 km), were subjected to processing directly after arrival (**ARV**) or where calves were allowed to rest (**REST**) for 24 h before initial processing. During the 24 h rest period, calves were housed in receiving pens (54 calves per pen) with free access to two 132-L water fountains (0.77 m^2 each; CATTLEMASTER 840; Ritchie Inc., Conrad, IA) centrally located on the fence line of neighboring pens, a round bale of long-stem wheat hay, and a commercially available feedlot receiving diet (**RAMP**; Cargill Inc., Dalhart, TX).

At initial processing, heifers were individually weighed using a Daniels Bud Box System (Model: AH-10; Ainsworth, NE), tagged with a unique identification and pen tag, dewormed (Safe-guard; Intervet Inc.; Millsboro, DE), and vaccinated. Vaccination protocol included a modified live virus vaccine for the control of IBR, BVD type 1 and 2, PI3, bovine respiratory syncytial virus (**BRSV**), *Manheimia haemolytica* and *Pasteurella multocida* (Vista Once SQ; Intervet Inc., Merck Animal Health; Omaha, NE), and a bacterial vaccination for the control of clostridial diseases (Vision 7, Intervet Inc., Merck Animal Health). Calves were revaccinated on d 14 with the vaccines described above. The receiving protocol did not include metaphylactic antibacterial treatment.

Calves were housed in soil-surfaced pens (16 pens; 12 m × 35 m; 11 m bunk space) for the remainder of the 56-d experimental period with access to a 76-L water fountain (CATTLEMASTER 480; Ritchie Inc.). All heifers were fed a receiving diet (RAMP; 19.9% CP, NE_m =1.72 Mcal/kg, NE_g =1.11 Mcal/kg) twice daily at 0700 and 1300 h using a feed truck with 6 individually fitted bins and horizontal augers for dispensing the feed. Unconsumed feed in bunks were evaluated daily at 0615, 1230, and 1830 h, and information obtained were used to manage feed delivery to have trace amount of feed at 1830 h with no feed at 0700 h. Orts remaining at 0645 h were obtained for DM analysis (100°C for 24 h) to adjust daily DMI accordingly. Representative samples of each load of receiving ration were obtained for nutrient analysis by a commercial laboratory (Servi-Tech Laboratories; Amarillo, TX).

Management and Collections

On d 1, 2, 3, and 28, individual BW and rectal temperature (GLA M700; GLA Agricultural Electronics, San Luis Obispo, CA) were obtained for all calves. Blood samples (via jugular venipuncture) for all calves were collected on d 1, 2, 3, and 14. Calves assigned to ARV had their BW, rectal temperature, and blood samples collected upon arrival (d 0) when REST calves were allowed to rest before initial processing (d 1). Pen weights were obtained on all pens of calves on d 56. Blood samples were used to determine the hydration status (hematocrit) of calves upon arrival and the effect of rest as part of the receiving protocol on rehydration. Hematocrit values were measured shortly after blood collection using capillary tubes (Plastic Capillaries for Hematocrit; Innovative Med Tech, Leawood, KS) sealed with a clay-like material (Critoseal; St. Louis, MO) centrifuged at 12,000 × *g* for 5 min (Micro Hematocrit Centrifuge, Model M24; LW Scientific Inc., Lawrenceville, GA).

Clinical assessment of calf health was performed daily based on depression, anorexia, respiration, and temperature (**DART**; 3-point scale method). Calves showing signs of morbidity based on the DART criteria were removed from their home-pens and taken to the handling facilities for further evaluation. Subsequently, calf BW and rectal temperature were measured and calves were given severity scores of 0

to 3 for depression and respiration. Calves warranted medical treatment if they had not gained weight since previous recording, had a rectal temperature greater than 40.5°C, or had a severity score of 2 or higher for depression or respiration. The first medical treatment consisted of an antibiotic (florfenicol) and a fast-acting non-steroidal anti-inflammatory (flunixin meglumine) combination as a single dose (Resflor Gold; Merck Animal Health, Summit, NJ). Calves warranting a second medical treatment received a broad-spectrum antibiotic (crystalline free acid of centioflur) active against gram-negative bacteria (Excede; Zoetis Inc.; Kalamazoo, MI). The third medical consisted of an oxytetracycline antibiotic (Bio-Mycin 200: Boehringer Ingelheim Vetmedia, Inc., St. Joseph, MO). Heifers showing sign of morbidity after the third medical treatment were permanently removed from the study.

Statistical Analysis

Calf performance and blood hematocrit values were analyzed as continuous variable using the MIXED procedure (SAS Inst. Inc., Cary, NC). Morbidity and mortality data were analyzed as categorical proportions using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC). The statistical model included the effect of treatment (receiving protocol) with block as random factor. The model included day as repeated measure with compound symmetry as covariance structure for analysis of blood hematocrit. Treatment differences were considered significant when $P < 0.10$ and a tendency when $P < 0.15$.

RESULTS

A tendency for a treatment × day interaction ($P = 0.12$) occurred for blood hematocrit percentage (Fig. 1); on d 1, 2, and 14 blood hematocrit percentages were not different, but were greater ($P < 0.05$) for REST than ARV calves on d 3 (38.2 vs. 37.1 ± 0.53%). Blood hematocrit was not different (treatment; $P = 0.42$) among receiving protocols, but was lower (day; $P < 0.01$) on d 14 than d 1, 2, and 3 (33.8 vs. 38.1, 37.4, and 37.7 ± 0.62%). By design, d 0 hematocrit values are not available for calves on the REST treatment (Fig. 1), as these calves were allowed rest upon arrival without being subjected to sample collection.

For calf morbidity, percentage of calves receiving a first and (or) second medical treatment was not affected ($P \geq 0.39$) by receiving protocol (Table 1). The total medical treatments tended to be greater ($P = 0.14$) for REST than ARV calves. Calf mortality was not different ($P = 0.99$) among receiving protocols; 7 of 112 calves died on each treatment group.

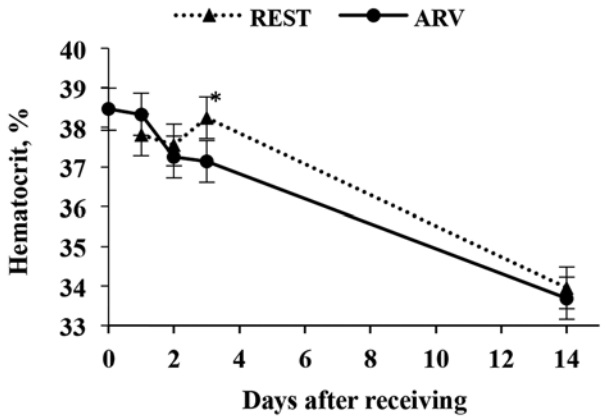

Figure 1. Blood hematocrit percentages of heifers during the first 14 d after receiving in response to initial processing upon arrival (ARV) or a 24 h delayed processing (REST). Effects were: treatment × day interaction ($P = 0.12$), day ($P < 0.01$), and treatment ($P = 0.42$). *Different from ARV ($P < 0.05$).

TABLE 1. The effects of receiving protocol on health and performance of newly received feedlot heifers

	Treatment[1]			
Item	ARV	REST	SEM	*P*-value
Pens[2]	8	8		
BW, kg				
d 1	185.9	187.4	1.23	0.37
d 28	201.2	199.5	1.85	0.53
d 56	239.1	236.1	2.40	0.40
DMI, kg/d				
d 1 to 28	3.43	3.42	0.11	0.97
d 28 to 56	6.14	5.95	0.13	0.32
d 1 to 56	4.79	4.69	0.09	0.46
ADG, kg/d				
d 1 to 28	0.57	0.45	0.05	0.13
d 28 to 56	1.35	1.31	0.05	0.50
d 1 to 56	0.97	0.89	0.03	0.12
G:F				
d 1 to 28	0.165	0.131	0.014	0.10
d 28 to 56	0.220	0.220	0.007	0.99
d 1 to 56	0.201	0.188	0.005	0.12
Morbidity,[3] %				
First treatment	42.86	47.32	4.71	0.51
Second treatment	15.15	19.62	4.27	0.39
Third treatment	2.68	3.57	1.75	0.71
Total treatment	60.71	70.54	4.61	0.14
Mortality,[4] %				
d 0 to 56	6.25	6.25	2.28	0.99

[1]Receiving protocol where calves received initial vaccines on the day of arrival (ARV), or where calves were allowed rest (REST) for 24 h with free access to water, long stem wheat hay and a receiving ration (Ramp; Cargill Inc., Dalhart, TX) before receiving their initial vaccines.

[2]Soil surface pens with 14 calves per pen.

[3]First treatment = percentage of first medical treatment of calves, Second treatment = percentage second medical treatment of calves, Third treatment = percentage third medical treatment of calves, and Total treatment = percentage of total medical treatment (first, second, and third medical treatment combined.

[4]Mortality = percentage of calves that died during the 56 d trial period.

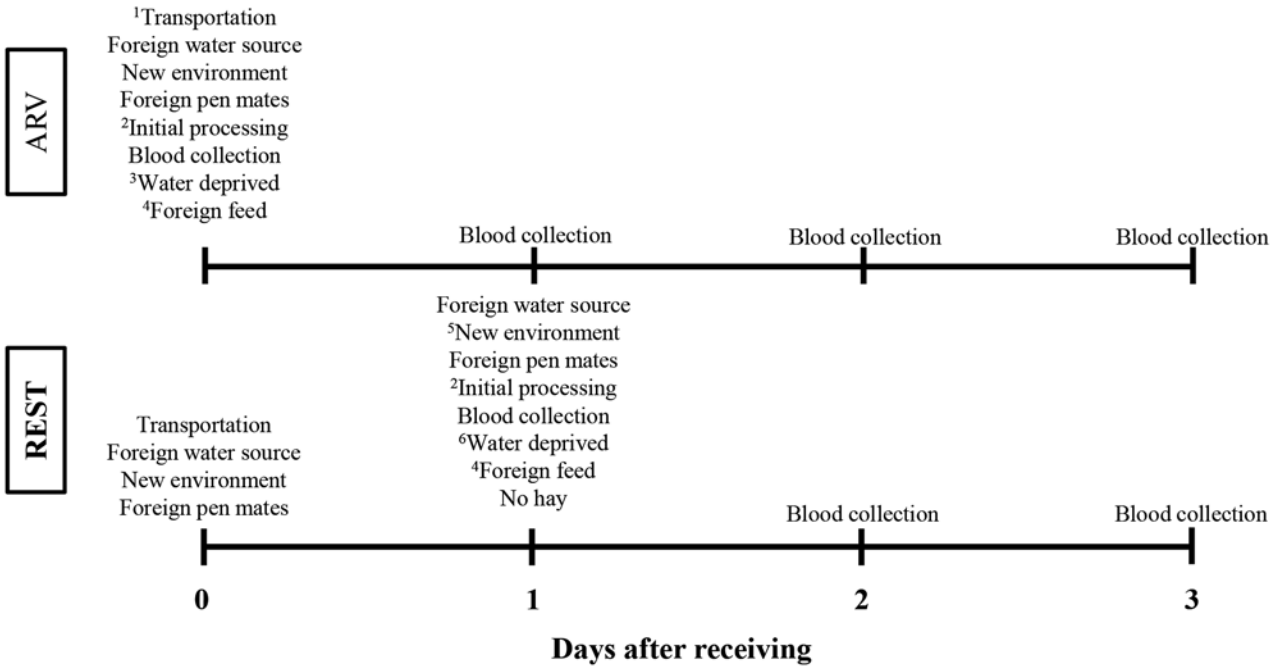

Figure 2. Stress factors calves are exposed to during the receiving period. [1]Transportation: 12 h on truck; 1,159 km; [2]Initial processing: weighed, ear tagged with a unique identification and pen tag, dewormed, and vaccinated; [3]No access to water on truck or during initial processing; [4]Receiving ration (RAMP; Cargill Inc.); [5]Moved from receiving to new home pen after processing; [6]During initial processing kept in sorting pens without access to water.

Body weights of calves on d 1, 28, and 56 were not different ($P \geq 0.37$) among receiving protocols (Table 1). Receiving protocol did not affect calf DMI ($P \geq 0.32$). From d 1 to 28, REST calves tended to have a lower ADG ($P = 0.13$) and G:F ratio ($P = 0.10$) than ARV calves. From d 28 to 56, heifer ADG and G:F were not different ($P \geq 0.50$) among receiving protocols. From d 1 to 56, REST calves tended have a lower ADG ($P = 0.12$) and G:F ($P = 0.12$) than ARV calves.

DISCUSSION

Hydration Status

Newly received feedlot calves, subjected to long periods of water deprivation during marketing and transportation, have been reported to be dehydrated (Parker et al., 2007; Richeson et al., 2013). Elevated hematocrit percentages (38.5%) for ARV calves on d 0 were indicative of dehydration, as they were greater than the values (25 to 33%) considered normal (UC Davis College of Veterinary Medicine, 2011; Cornell University College of Veterinary Medicine, 2014). In attempt to replace lost body water, REST calves were allowed 24 h of rest with free access to water, wheat hay and a receiving ration. However, no hematocrit differences between REST and ARV calves on d 1 (24 h after arrival) suggest that 24 h of rest might not be sufficient to replace lost body water.

We hypothesized that rest upon arrival with access to water would improve hydration status of calves which would be evident in lower hematocrit values. Greater blood hematocrits for REST than ARV calves on d 3 is in contrast with what was expected and may have been attributed to the suppressive effect of cortisol on a principal mechanism of resistance to dehydration. Parker et al. (2004) reported that in the presence of water deprivation and stress, excess cortisol has a suppressive effect on the renin-angiotensin-aldosterone axis responsible for water reabsorption from the kidneys. Calves allowed rest upon arrival are subjected to many new stressors on d 1 (Fig. 2) in addition to initial processing. These stressors are in excess of ARV calves and include a new pen environment with foreign pen mates, water source and feed (receiving ration without access to hay as upon arrival), and could result in suppressed feed and water intake, and elevated blood cortisol levels. This is consistent with greater hematocrits (more dehydrated) observed for REST calves on d 3 and similar to ARV hematocrits observed on d 0.

Animal Health and Performance

Bovine respiratory disease predominantly starts with a primary viral infection as normal immune barriers are weakened, and predisposes the animal to secondary bacterial infection (Duff and Galyean, 2007). Such immune barriers include the respiratory mucus and precilliary layer responsible for the clearance of inhaled pathogens. Stressed dehydrated calves have decreased water content in respiratory mucus, and increased glucocorticoid secretion which decreases the ciliary mo-

tility in the trachea permitting pathogens invasion (Constantinopol et al., 1989; Jones et al., 1989). Once these immune barriers are crossed the body relies on antibody response to provide the ultimate defenses against the invading pathogen. Kegley et al. (1997) reported decreased antibody responses for calves subjected to long periods of transportation. This suggests that dehydrated calves could be more susceptible to respiratory disease. Stressed morbid calves are known to have depressed feed intake (Preston, 2007) which are exacerbated by the reluctance of dehydrated cattle to eat (Noffsinger et al., 2015). Adequate water intake at arrival to replenish lost body water is critical to subsequent feed intake, health, and performance (Brew et al., 2011).

In this study, we hypothesized that rest upon arrival with access to water would allow calves to rehydrate and subsequently improve health and performance. Improved performance may be due to the anticipated greater DMI of healthier calves with less energy expenditure on immune responses. However, due to inability of calves to replace the lost body water during the 24 h rest period, the anticipated health and performance benefits were not observed. No morbidity or mortality differences among calves suggest that greater hematocrits for REST calves observed on d 3 were not sufficient to have a lasting effect on animal health. A tendency for a lower ADG and G:F for REST than ARV calves was observed and could, in part, be attributed to the improved hydration status of ARV calves on d 3. Brew et al. (2011) reported greater gains for more hydrated calves with greater water intakes.

These results imply that 24 h of rest after a 12-h transportation episode is not enough time to allow calves to sufficiently rehydrate to observe health and performance benefits. Postponing initial processing delays exposure to many stress factors and may cause dehydration to the same extent as 12 h of transportation.

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Influence of dried distiller's grains with solubles on spermatozoa morphological abnormalities of ram lambs

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Abstract: The inclusion of dried distiller's grains (DDGS) at 15%, 30%, and 45% of the ration was hypothesized to have an increasing linear effect on spermatozoa morphological abnormalities in growing ram lambs. Following the removal of DDGS from the ration, we hypothesized that the ram lambs would recover and become reproductively sound, independent of treatment. To test this hypothesis, Suffolk and Hampshire ram lambs (n =112) were allocated to four treatments (n = 4 pens/treatment; 7 rams/pen) in a completely random design. Dietary treatments were 60% corn, 25% oats, and 15% commercial market lamb pellet (CON), 15% of the ration as DDGS substituted for corn (% DM basis; 15DDGS), 30% of the ration as DDGS substituted for corn (% DM basis; 30DDGS) and 45% of the ration as DDGS substituted for corn (% DM basis; 45DDGS). Rams were fed to d 112 on their respective treatment (PHASE 1), after which rams were placed on the CON ration until d 168 (PHASE 2). Semen samples were collected on a subset of 64 rams (4 rams/pen) to evaluate semen quality on d 84, 112, 140, and 168. Semen samples were analyzed for morphological abnormalities, both major and minor defects. A treatment × PHASE interaction (P = 0.05) was observed for the occurrence of distal droplets along the tails of the spermatozoa. This defect is considered minor and can lead to subfertility in natural breeding situations. The only morphological abnormality effect observed in PHASE 1 was linear decrease (P = 0.04) in the occurrence of strongly folded tails as the inclusion of DDGS increased in the diet. An overall linear decrease (P = 0.02) was observed for the occurrence of bent heads on the sperm as DDGS increased in the diet. The cause of this defect is unknown; however, it can be detrimental to fertility and embryonic development. A day effect was observed (P < 0.001) for the occurrence of normal spermatozoa, with both of the PHASE 2 collections having a greater occurrence than the first and third collection days. Based on the means of the collection days, ram lambs would have failed a breeding soundness exam on the first two collection days (d 84 and 112). Increasing DDGS in the diet had no negative effects on ram lamb reproductive traits. Additional research is needed to elucidate the causes of these results.

Key words: dried distiller's grains with solubles, rams, reproductive traits, semen morphology
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INTRODUCTION

Ethanol production in the United States continues to increase exponentially (Renewable Fuels Association, 2015). The byproduct of the ethanol industry, dried distiller's grains with solubles (**DDGS**), provides an affordable and viable feed source for livestock, especially ruminants. Dried distiller's grains are readily incorporated into diets to supplement RUP as DDGS have much more available protein when compared to corn. Although, it is important to consider that DDGS can be high in sulfur and crude fat. New generation ethanol refineries have decreased the variability in crude fat and protein, however mineral content, especially S, can still be highly variable (Spiehs et al., 2002). Sulfur content is a concern in ruminant diets due to the possibility of inducing polioencephalomalacia (**PEM**). In feedlot lambs, DDGS have been included in the ration at rates of up to 60% with no negative effects on performance (Schauer et al.,

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2008; Neville et al., 2011). With the growing popularity of feeding DDGS by the sheep industry, research is needed that investigates the possible impacts of DDGS on ram fertility. Van Emon et al. (2013) reported a linear decrease in spermatozoa concentration as DDGS increased in the diet. The feedlot growth performance data for the present trial indicated increasing concentrations of DDGS in growing ram lamb rations may actually increasing weight gain, but did not negatively affect spermatozoa concentration (Crane et al., 2015). The current trial tested the hypothesis that ram lambs consuming increasing concentrations of DDGS in the ration would have declining reproductive traits. We also hypothesized that ram lambs would reproductively convalesce once being removed from diets containing DDGS. The objectives used to test this hypothesis were to determine the influence of increasing concentrations of DDGS in ram lamb rations on ram lamb reproductive traits.

MATERIALS AND METHODS

All procedures were approved by the animal care and use committee of North Dakota State University (protocol # A14060). This study was conducted at the North Dakota State University Hettinger Research Extension Center in Hettinger, ND.

Reproductive Trial

Ram lambs (Suffolk and Hampshire) were purchased from four producers in North and South Dakota, Minnesota, and Iowa. Prior to purchase, ram lambs were vaccinated for *Clostridium perfringens* types C and D and tetanus, weaned at 60 d of age, and revaccinated. At approximately 90 d of age (May of 2014), rams were purchased and transported to the Hettinger Research Extension Center. Ram lambs were adapted to a 60% corn, 25% oats, and 15% commercial market lamb pellet diet (CON; DM basis) for approximately 2 weeks. Ram lambs (n=112) were stratified by weight (48.7 ± 0.31 kg) and breed and randomly assigned to 1 of 16 outdoor pens (7 rams/pen; 18 m^2/ram). Pens were assigned randomly to 1 of 4 treatments in a completely random design to 1 of 4 treatments, with pen serving as the experimental unit (n = 4 pens/treatment). Dietary treatments were 60% corn, 25% oats, and 15% commercial market lamb pellet (**CON**), 15% of the ration as DDGS substituted for corn (% DM basis; **15DDGS**), 30% of the ration as DDGS substituted for corn (% DM basis; **30DDGS**) and 45% of the ration as DDGS substituted for corn (% DM basis; **45DDGS**) as described in Table 1. Study diets were balanced to be equal to or greater than the CP and TDN requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007) and to maintain a Ca:P ratio of 2:1 or greater. Rations were ground (1.25 cm screen) and mixed in a grinder-mixer (GEHL mix-all, Model 170; West Bend, WI) and were provided ad libitum to ram lambs via bulk feeders (70 cm bunk space/ram). Ram lambs had continuous access to clean, fresh water. Feeders were checked daily and cleaned of contaminated feed (fecal contamination, wet feed due to precipitation, etc.). Ram lambs were weighed on two consecutive d at the beginning (d 0 and 1) and the end of the trial (d 167 and 168) and weighed once every 28 d. Ram lambs were fed their respective treatments until d 112 (**PHASE 1**) and on d 112 all feed was removed from self-feeders and pens were reallocated to the CON diet until d 168 (**PHASE 2**). Sixty-four ram lambs (a subsample of the 112 ram lambs; 4 ram lambs/pen; 16 rams/treatment; n = 4) were chosen for semen morphology analysis. Two of the four ram lambs in each pen were selected based on weight and breed to provide a representative

TABLE 1. Ingredient and nutritional composition of diets fed to growing ram lambs (DM basis)

	Dietary treatment[1]			
Item	CON	15DDGS	30DDGS	45DDGS
Ingredient, %				
DDGS[2]	-	14.93	29.7	44.33
Corn	60	44.78	29.7	14.78
Oats	25	24.88	24.75	24.63
Lamb Pellet[3]	15	14.93	14.85	14.78
$CaCO_3$	-	0.48	1.00	1.48
Nutritional composition, % DM				
Ash	4.00	4.56	5.18	5.66
TDN[4]	86.53	84.83	83.06	81.44
CP	16.42	19.89	23.32	26.74
ADF	6.56	7.88	9.16	10.59
Crude Fat	3.52	4.16	4.79	5.42
S, %	0.21	0.27	0.32	0.37
P, %	0.41	0.51	0.61	0.71
K, %	0.74	0.90	1.05	1.21
Mg, %	0.18	0.23	0.27	0.31
Ca, %	0.68	1.18	1.69	2.18
Ca:P	1.65	2.31	2.77	3.07
Na, %	0.19	0.24	0.29	0.34
Mn, mg/kg	109	117	125	133

[1]Diets (DM basis) were balanced to be equal to or greater than CP and energy requirements of a 40 kg ram gaining 300 g/d (NRC, 2007). Treatments were CON: 60% corn, 25% oats, and 15% commercial market lamb pellet, 15DDGS: 15% DDGS substituted for corn (DM basis), 30DDGS: 30% DDGS substituted for corn (DM basis), and 45DDGS: 45% DDGS substituted for corn (DM basis).

[2]DDGS= dried distiller's grains with solubles.

[3]Commercial market lamb pellet contained 0.22 g/kg chlortetracycline, 38.0% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2 mg/kg Se, 52,863 IU/kg vitamin A, 5,286 IU/kg vitamin D, and 209 IU.

[4]Calculated.

subset for morphology analysis. Semen was collected on d 84, 112, 140, and 168 of the study via electroejaculation. Spermatozoa concentration was evaluated using a 20 μL subsamples of semen diluted in 3,980 μL of 3% NaCl solution and placed on a hemocytometer and assessed via microscope at 430x magnification. The dilution rate was 1:200, the hemocytometer factor was 50, and the conversion factor (converted units to spermatozoa/cm^3, or mL) was 1,000. Percent of specific morphological abnormalities of the diluted spermatozoa in a Trypan blue stain was determined on a pre-warmed glass slide at 40x magnification. The morphological abnormalities observed were: distal droplets, proximal droplets, tailless heads, abaxial tail implantation, abnormal acrosomes, simple bend in tails, narrow or small heads, bent heads, pyriform heads, strongly folded tails, mid-piece defects, maldeveloped spermatozoa, or normal spermatozoa.

Sampling and Laboratory Analysis

Ground ration samples were collected every 28 d (approximately 2.0 kg) and dried at 55°C for 48 h (The Grieve Corporation, Round Lake, IL) to determine DM. Orts were collected and weighed on d 112 and 168 of the trial and dried at 55°C for 48 h to determine DMI for PHASE 1 and 2, respectively. Dietary and ort samples were ground to pass a 2-mm screen (Wiley Mill; Arthur H. Thomas Corp., Philadelphia, PA) and shipped to a commercial lab (Midwest Laboratories, Inc., Omaha, NE) for proximate and mineral analysis (Table 1). Samples were analyzed for DM (method 930.15; AOAC Int., 2009), N (method 990.03; AOAC Int., 2009), NDF (Van Soest et al., 1991) as modified by Ankom Technology (Fairport, NY) using and Ankom 200 Fiber Analyzer without sodium sulfide, with amylase, and without ash corrections as sequentials, ADF (Goering and Van Soest, 1970), crude fat (method 945.16; AOAC Int., 2009), and minerals (inductively coupled atomic plasma and wet digest procedure).

Statistical Analysis

Ram lamb spermatozoa morphology traits were analyzed as a randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen serving as the experimental unit. The fixed effect included in the model was dietary treatment with the random effect of pen nested within treatment. The random effect of day was used in the REPEATED measures analysis for spermatozoa morphology. The model included the fixed effects of dietary treatment, PHASE, and treatment × PHASE. Random effects included pen nested within treatment, ram × pen × treatment, and PHASE × pen × treatment. Preplanned comparisons of linear, quadratic, and cubic contrasts were used to partition treatment effects. Significance was determined at $P \leq 0.05$. All interactions that were not clearly significant ($P \geq 0.20$) were removed from the model. To partition PHASE effects and treatment × PHASE interactions, least squares means were used ($P \leq 0.05$).

RESULTS AND DISCUSSION

The only treatment × PHASE interaction ($P = 0.05$) observed was for the occurrence of distal droplets along the tails of the spermatozoa. This interaction was observed on d 84 sampling in rams (PHASE 1) receiving the 15DDGS dietary treatment, with them having increased occurrence compared to other treatments and the other days. Means for this interaction were as follows: 0DDGS, 30DDGS, and 45DDGS for all four collection days were 0%, 15DDGS for d 84 collection was 16.25%, and 15DDGS for all other collection days were 0%. Distal cytoplasmic droplets are considered a minor defect, however, in natural breeding situations, have long been considered a cause of subfertility (Barth and Oko, 1989).

There was no treatment × PHASE, PHASE, or treatment effects ($P > 0.12$; Table 2) for abaxial tail implantation, abnormal acrosomes, and simple tail bends or coils. Proximal cytoplasmic droplets are classified as a major defect to spermatozoa, causing significant impairments to fertility. In this trial, while there was no effect of treatment on proximal cytoplasmic droplets ($P \geq 0.33$), a PHASE effect ($P < 0.001$) was observed, decreasing from d 84 throughout the trial to d 168, with the last three collections having similarly low percentages of the defect. This defect has been linked to poor cleavage rates of embryos (Amann et al., 2000; Thundathil et al., 2001). This is likely due to the ram lambs maturing throughout the trial, leading to the disappearance of the droplets. However, in trials observing Cu deficiencies in rams, when feeding Mo and high sulfate supplements, rams exhibited small percentages of droplets (1-4%; Van Niekerk and Van Niekerk, 1989).

Excessive sulfur intake can be toxic and could cause decreased performance and possibly cause PEM and death (Gould, 1998). No cases of PEM were observed in the present study (Crane et al., 2015) nor in the trial conducted by Van Emon et al. (2013). The NRC (2007) states that the maximum level of sulfur tolerated in concentrate diets is 0.3% of DM. However, in the present study, both the 30DDGS and 45DDGS diets were above this maximum level (Table 1). According to the NRC (2007), high dietary levels of sulfur can also alter selenium and copper utiliza-

TABLE 2. Effects of dried distiller's grains with solubles (DDGS) on spermatozoa morphology of growing ram lambs

	Dietary treatment[1]					*P*-value[3]		
Item, %	CON	15DDGS	30DDGS	45DDGS	SEM[2]	Linear	Quadratic	Cubic
Overall distal droplets	0.19	4.25	0.44	0.31	1.28	0.55	0.11	0.05
PHASE One	0.38	8.50	0.88	0.63	2.79	0.59	0.15	0.07
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall proximal droplets	1.00	1.75	0.94	1.00	0.56	0.74	0.54	0.33
PHASE One	2.00	3.50	1.88	2.00	1.22	0.77	0.57	0.38
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall tailless heads	13.44	15.12	13.31	10.88	2.88	0.46	0.48	0.82
PHASE One	20.00	20.25	21.63	15.88	5.28	0.65	0.57	0.73
PHASE Two	6.88	10.00	5.00	5.88	2.28	0.44	0.63	0.18
Overall abaxial tail implantation	0.19	0.88	0.13	<0.01	0.42	0.48	0.33	0.27
PHASE One	0.38	1.75	0.25	<0.01	0.83	0.48	0.33	0.27
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall abnormal acrosome	<0.01	0.06	0.13	0.44	0.20	0.12	0.53	0.78
PHASE One	0.00	0.13	0.25	0.88	0.39	0.12	0.52	0.77
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall simple bend or coil tails	9.06	7.13	10.56	7.94	1.96	0.99	0.86	0.19
PHASE One	9.13	9.88	13.88	10.63	3.07	0.54	0.52	0.45
PHASE Two	9.00	4.38	7.25	5.25	2.27	0.41	0.57	0.23
Overall narrow, small, or giant heads	0.44	0.38	0.38	0.94	0.54	0.54	0.57	0.84
PHASE One	0.88	0.75	0.75	1.88	1.10	0.55	0.57	0.84
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall bent heads	3.25	1.06	1.56	1.00	0.60	0.02	0.18	0.17
PHASE One	1.63	1.13	2.75	1.63	0.70	0.61	0.66	0.13
PHASE Two	4.88	1.00	0.38	0.38	0.94	0.002	0.05	0.54
Overall pyriform heads	0.44	0.50	0.25	0.19	0.22	0.32	0.78	0.62
PHASE One	0.88	1.00	0.38	0.38	0.43	0.27	0.88	0.48
PHASE Two	0.00	0.00	0.13	<0.01	0.06	0.66	0.33	0.19
Overall strongly folded tails	1.63	0.38	0.56	0.06	0.47	0.04	0.43	0.31
PHASE One	3.25	0.75	1.13	0.13	0.97	0.04	0.45	0.34
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall mid-piece defects	0.19	0.25	0.56	0.06	0.20	0.94	0.16	0.23
PHASE One	0.38	0.50	1.13	0.13	0.38	0.94	0.15	0.22
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall maldeveloped heads	0.69	0.25	0.19	0.38	0.24	0.35	0.20	0.91
PHASE One	1.38	0.50	0.38	0.75	0.52	0.40	0.241	0.92
PHASE Two	0.00	0.00	0.00	0.00	-	-	-	-
Overall normal morphology	70.69	68.94	72.25	77.75	3.95	0.17	0.36	0.87
PHASE One	61.50	53.13	56.63	66.25	7.16	0.58	0.22	0.86
PHASE Two	79.88	84.75	87.88	89.25	3.28	0.04	0.60	1.00

[1]Diets (DM basis) were balanced to be equal to or greater than CP and energy requirements of a 40 kg ram gaining 300 g/d (NRC, 2007). Treatments were CON: 60% corn, 25% oats, and 15% commercial market lamb pellet, 15DDGS: 15% DDGS substituted for corn (DM basis), 30DDGS: 30% DDGS substituted for corn (DM basis), and 45DDGS: 45% DDGS substituted for corn (DM basis).

[2]$n = 4$.

[3]*P*-value for linear, quadratic, and cubic effects of increasing DDGS.

tion and absorption. Van Emon et al. (2013) indicated inadequate utilization of selenium and copper as a possible explanation for the observed reduction in spermatozoa concentration. The percentage of tailless heads also exhibited a day effect ($P < 0.001$) decreasing from PHASE 1 to PHASE 2, which each collection within the phases being similar to one another. Others have observed that this defect is usually due to testicular hypoplasia or degeneration, sexual inactivity, or more specific conditions, like decapitated sperm defect (Barth and Oko, 1989). In this trial, the presence of this defect was likely caused by sexual inactivity as the ram lambs were not exposed to females from approximately d 90 of age until trial conclusion. The presence of narrow, small, or giant heads of the sperm exhibited a day effect ($P = 0.02$) decreasing from the

first collection to the last three collection days, while not exhibiting a treatment effect ($P \geq 0.54$). In the previously mentioned Cu deficiency trial, this defect was moderately observed (6-12%) in rams that were Cu deficient as a result of Mo and sulfate supplementation (Van Niekerk and Van Niekerk, 1989).

An overall linear decrease ($P = 0.02$) was observed for the occurrence of bent heads on the sperm as DDGS increased in the diet. Although the cause of this defect is not known, it can be detrimental to fertility as it can impair fertilization rate, embryonic development, and failure of cleavage (Menon et al., 2011). A day effect was observed ($P = 0.02$) for the occurrence of pyriform shaped heads, with PHASE 1 collections having greater occurrence than PHASE 2, with no difference ($P \geq 0.32$) due to treatment. Similar to the other sperm head defects, pyriform shaped heads impair the spermatozoa's ability to fertilize the oocyte efficiently and effectively. A day effect was also observed ($P = 0.009$) for the defect of strongly folded or coiled tails. There was also a linear decrease ($P = 0.04$) in the occurrence of this defect as the inclusion of DDGS increased in the ration. This is interesting as the Cu deficiency trial recorded this defect as one of the most prevalent (18-25%) when Cu was deficient (Van Niekerk and Van Niekerk, 1989), leading one to explicate that the S present in the DDGS in the present trial must not be leading to mineral deficiencies *in vivo*. Similar day effects were observed ($P < 0.05$) for mid-piece defects as well as maldeveloped heads. Both exhibited a decrease in occurrence from PHASE 1 collections to PHASE 2 collections, with no difference due to treatment ($P \geq 0.16$). This is once again most likely due to maturation of the ram lambs, testicles, and increases in sexual activity.

A day effect was observed ($P < 0.001$) for the occurrence of normal spermatozoa, with both of the PHASE 2 collections having a greater occurrence than the first collection day and the d 140 collection (3rd collection) being greater than the first collection as well. Based on the means of the collection days, ram lambs would have failed a breeding soundness exam on the first two collection days (d 84 and 112). However, there was no treatment effect ($P \geq 0.17$) on the occurrence of normal sperm. Interestingly, although there was no treatment effect, the means for the treatments should be taken into consideration. Although the measurements of the young rams brought down the mean values, the 0DDGS, 15DDGS, and 30DDGS treatments would have caused the rams to fail a breeding soundness exam as satisfactory percentages are listed as ≥ 75% normal spermatozoa according to Ley et al. (1990). This data suggests that supplementing growing ram lambs with a high energy and protein feed, such as DDGS, might decrease the time before young rams can effectively breed. Van Emon et al. (2013) did not report spermatozoa morphology, however, they did report that spermatozoa concentrations decreased linearly as DDGS concentrations in the diets increased. Exactly what is causing the observed affects is not known. Dried distillers grains with solubles possesses multiple qualities that should be considered, such as CP, crude fat, and sulfur. However, the current trial showed no negative impacts on reproduction in ram lambs being fed increasing DDGS; therefore, more research is needed to elucidate the conflicting results and what the possible cause is. Previous research on human sperm revealed semen samples with low sperm concentrations, high incidence of abnormal sperm morphology, and diminished fertility had higher sperm creatine phosphokinase (**CK**) activity (Huszar and Vigue, 1993). Higher CK activity was related to increased content of CK and other proteins in the sperm resulting in those sperm heads being significantly larger and rounder, with increased morphological irregularities and increased cytoplasm believed to be due to failure of spermatogenesis (Huszar and Vigue, 1993). This trial concluded that higher CK activity results in cellular immaturity and a failure to complete spermatogenesis (Huszar and Vigue, 1993). Potentially the increased CP in both our trial and that of Van Emon et al. (2013), as a result of DDGS increasing in the ration, is contributing to the negative effects on sperm quality. Additional research is needed to ascertain why these effects are occurring.

IMPLICATIONS

Dried distiller's grains with solubles had no negative impacts on the morphology of spermatozoa, and for some traits, improved morphology in ram lambs when fed at up to 45% of the ration. However, producers should be aware of the possibility of sulfur toxicity and polioencephalomalacia when feeding dried distiller's grains with solubles at more than 15% of the ration. We are only aware of two trials elucidating reproductive quality of rams when feeding dried distiller's grains with solubles in the diet. More research is needed in the area of nutritional effects on ram fertility to further elucidate what is causing the observe effects. Future research should focus on specific nutrient and mineral concentrations, as well as heavy metals and their effects on both ram and ewe fertility.

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Supplemental glycerin alters rumen fermentation and in situ degradation in steers exposed to an endotoxin[1]

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ABSTRACT: Stress decreases DMI and may negatively impact rumen fermentation and degradability of nutrients. Crude glycerin supplementation may increase dietary energy intake. This study evaluated the effects of glycerin in drinking water on DM degradability and rumen fermentation in 24 ruminally-cannulated beef steers (203 ± 3.8 kg BW) exposed to lipopolysaccharide (LPS). Treatments were a 2 × 2 factorial of 0 vs. 25 g/L glycerin (-GLY vs. +GLY) in drinking water and 0 vs. 3 µg LPS (-LPS vs. +LPS) per kg of BW. Steers were housed in a metabolism facility for 12 d (7-d adaptation and 5-d collection). On d 8, LPS treatments were injected subcutaneously. In situ bags were incubated in the rumen for 2, 4, 8, 12, 18, 24, 36, 48, and 72 h after LPS for calculation of ruminal DM degradation. Ruminal fluid for pH, and VFA and NH_3 analysis was collected at -2, 0, 2, 4, 8, 12, 18, 24, 36, 48, and 72 h. The extent of DM degraded in the rumen was not different between −GLY and +GLY for −LPS steers, but was lower when +LPS steers received +GLY vs. –GLY (LPS × GLY; $P = 0.07$). The degradation rate of DM was also not different when −LPS steers received +GLY or −GLY, but was lower when +LPS steers received +GLY vs. –GLY (LPS × GLY; $P < 0.01$). A GLY × h interaction ($P \leq 0.05$) occurred for ruminal pH, acetate, propionate, and acetate:propionate (A:P) ratio. Ruminal pH of steers was lower for +GLY than –GLY at 4 and 12 h, and not different at all other hours sampled. Acetate of +GLY steers decreased from -2 to 4 h and was lower for +GLY than −GLY steers, whereas propionate of +GLY steers increased from -2 to 4 h and was greater for +GLY than −GLY steers, resulting in a lower A:P ratio for +GLY than −GLY steers. An LPS × h interaction ($P \leq 0.01$) occurred for ruminal NH_3 and butyrate. Ruminal NH_3 was not different between LPS steers from -2 to 18 h, but were lower for +LPS than –LPS steers from 24 to 72 h. Butyrate increased from 0 to 8 h and was greater for +LPS vs. –LPS steers at 2, 4, 8, 12, and 18 h. This research suggests glycerin supplementation in drinking water alters rumen fermentation and DM degradation in steers exposed to LPS.

Key words: cattle, glycerol, in situ, lipopolysaccharide
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INTRODUCTION

Stressed calves that have been transported to a feedlot typically have reduced DMI, which may limit energy intake needed to support the immune system (Duff and Galyean, 2007). Crude glycerin is a liquid byproduct from the biodiesel industry that has been used in cattle diets as a source of supplemental dietary energy (Parsons et al., 2009). Providing a high-energy product such as glycerin may alleviate the effects of a compromised immune system in stressed receiving calves, particularly if supplemented via drinking water to overcome consumption limitations associated with low DMI of stressed cattle (Carey et al., 2017). In addition to reduced DMI, stress associated with transport, fasting, a new environment, or exposure to an endotoxin also affects gut motility, rumen fermentation, and overall rumen function (Galyean et al., 1981; Gilliam et al., 2009). Therefore, we hypothesized that glycerin supplementation via drinking water could provide a source of additional energy

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for microbial fermentation which may offset some of the negative effects of stress on rumen fermentation (Samuelson et al., 2016). The objective was to evaluate the effects of supplementing glycerin in drinking water on ruminal DM degradability and rumen fermentation characteristics of steers exposed to lipopolysaccharide (**LPS**).

MATERIALS AND METHODS

Animals and Facilities

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Twenty-four ruminally cannulated beef steers (203 ± 3.8 kg BW) were housed in individual tie stalls in a metabolism building. Steers were fed a corn grain and alfalfa hay-based diet (Table 1) in two equal portions at 0700 and 1900 h. Daily DMI was limited to 1.7% of BW to correspond with low intakes of newly received feedlot calves (NRC, 2000).

Experimental Design and Treatments

The experiment was a randomized complete block design. Steers were blocked by BW into 2 blocks with 12 steers per block. Within each block, steers were randomly assigned to treatments in a 2 × 2 factorial arrangement. Treatments were energy supplementation of either 0 or 25 g/L crude glycerin (**−GLY** vs. **+GLY**) added to the drinking water, and subcutaneous injection of 0 or 3 µg LPS per kg of BW (**−LPS** vs. **+LPS**; *Escherichia coli* O55:B5; Sigma Chem. Co., St. Louis, MO) dissolved in 2 mL of sterile saline. Steers were housed in the metabolism building for 12 d, which allowed 7 d for adaptation to facilities and diet and 5 d for collections. The LPS treatments were administered at 0900 h on d 1 of the collection period (d 8 of the experiment), and glycerin treatments were applied throughout the 12-d study.

Sample Collections and Analysis

A sample of the diet (Table 1) was dried in a forced-air oven (POM-326F, Blue M Electric Company, Blue Island, IL) at 55°C for 48 h, allowed to air-equilibrate, and ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen. Ground feed samples (5 g) were added to in situ Dacron bags (size 5 × 10 cm; pore size 50 µm; ANKOM Technology, Macedon, NY), which were sealed with an impulse heat sealer (AIE-200; American International Electric Sealer Supply, South El Monte, CA). In situ bags for each animal were then placed into nylon mesh bags (30.5 × 25.4 cm) and soaked in water (±39°C) for 20 min. After soaking, the nylon mesh bag containing all in situ bags (except for 0 h) and a plastic-coated weight (±100 g) were inserted directly into the rumen and allowed to incubate. At 2, 4, 8, 12, 18, 24, 36, 48, and 72 h after LPS administration, 2 in situ bags containing diet sample and 1 in situ bag containing no sample (blank) were removed from the rumen of each steer. The in situ bags were immediately rinsed with tap water to remove particulate matter, and then rinsed in cold water in a top-loading commercial washing machine (General Electric Company, Louisville, KY). Each set of in situ bags were washed for a total of 5 cycles, which included 1 min of agitation (delicate cycle), and 2 min of spin. Bags were then dried in a forced-air oven at 55°C for 48 h, allowed to air-equilibrate, weighed for determination of partial DM and stored at room temperature. In situ bags were later dried in a forced-air oven at 105°C for 48 h to determine final DM.

Approximately 30 mL of ruminal fluid was collected using a filtered suction strainer (Precision Machine Co, Lincoln, NE) and collection flask 2 h prior to (-2 h) and immediately before LPS injection (0 h), then at 2, 4, 8, 12, 18, 24, 36, 48, and 72 h after LPS injection. Immediately after collecting the rumen fluid, pH was recorded using a portable pH meter (Mettler Toledo, Schwerzenbach, Switzerland) and ruminal fluid samples were placed on ice to cease fermentation and then frozen (-20°C) until analysis. Ruminal fluid

TABLE 1. Diet composition

Item	DM basis
Ingredient, %	
Corn grain, cracked	35.8
Alfalfa hay	30.0
Dried distiller's grains	15.0
Beet pulp shreds with molasses	10.0
Molasses	8.0
Urea	0.50
Salt	0.30
Limestone	0.25
Vitamin supplement[1]	0.12
Trace mineral supplement[2]	0.02
Rumensin[3]	0.017
Nutrient,[4] %	
NDF	26.1
CP	15.1
Ca	0.75
P	0.33

[1]Supplied (DM basis) 3,000 IU/kg vitamin A, 600 IU/kg vitamin D, 150 IU/kg vitamin E.

[2]Contained 1.8% Cu, 9.0% Zn, and 360 ppm Se (Beefmax 510, Cargill Inc., Minneapolis, MN).

[3]Supplied 34 mg monensin per kg of DM (Elanco Animal Health, Indianapolis, IN).

[4]Analyzed by SDK Laboratories (Hutchinson, KS).

TABLE 2. Rumen degradable fractions and degradation rates of feed in steers exposed to lipopolysaccharide (LPS) and supplemented with glycerin (GLY)

	Treatments[1]							
	-LPS		+LPS			*P*-value		
Item	-GLY	+GLY	-GLY	+GLY	SEM	LPS × GLY	LPS	GLY
DM Fraction,[2] %								
A	39.4	39.4	39.6	39.2	2.46	0.42	0.96	0.37
B	36.4	38.3	37.8	34.7	2.12	0.07	0.41	0.65
C	24.2	22.4	22.7	26.1	1.38	0.07	0.43	0.58
DM *kd*, %/h	1.8[ab]	2.1[b]	2.1[b]	1.6[a]	0.11	<0.01	0.31	0.43

[a,b]Means with different superscript letters are different ($P \leq 0.05$).

[1]Treatments were subcutaneous injection of 0 or 3 µg LPS per kg of BW (−LPS vs. +LPS; *Escherichia coli* O55:B5; Sigma Chem. Co., St. Louis, MO) dissolved in 2 mL of sterile saline, and energy supplementation of either 0 or 25 g/L crude glycerin (−GLY vs. +GLY) added to the drinking water.

[2]Fraction A = soluble fraction (residue that disappeared from in situ bags at 0 h); fraction C = ruminally undegradable fraction (residue remaining in the in situ bags after 72 h); fraction B = intermediately degradable fraction (100%-A-C); DM *kd* = rate of degradation of B fraction.

samples were allowed to thaw at room temperature, centrifuged at 20,000 × g for 20 min at 25°C twice (5415 R; Eppendorf North America, Hauppauge, NY) and decanted twice before analysis of VFA and NH_3. Concentrations of VFA in ruminal fluid were analyzed using gas chromatography (Agilent 7890A; Agilent Technologies, Santa Clara, CA) as described by Goetsch and Galyean (1983). Analyses of NH_3 concentrations in ruminal fluid were conducted as described by Broderick and Kang (1980) adapted for a plate reader (BioTek Instruments, Winooski, VT).

Calculations

Disappearance of DM from in situ bags was used to determine 3 fractions (A, B, and C) of ruminal degradability. Residue that disappeared from in situ bags at 0 h was considered the soluble fraction (Fraction A), and residue remaining in the in situ bags after 72 h was considered the ruminally undegradable fraction (Fraction C). Fraction B was considered potentially degradable in the rumen, and was calculated by difference (100%-A-C). For fraction B, rate of degradation (k_d) was calculated by regressing the natural logarithm of the percentage of DM remaining against the hours (2, 4, 8, 12, 18, 24, 36, 48, 72 h) that in situ bags were incubated in the rumen.

Statistical Analysis

Data were analyzed as a randomized complete block design using MIXED models (SAS Inst. Inc., Cary, NC). Individual steer was the experimental unit. The fixed effects of the model for dependent variables without repeated observations (in situ degradability) included LPS, GLY, and LPS × GLY interaction with block as a random effect. The model for dependent variables with repeated measures (pH, VFA and NH_3) included LPS, GLY, hour, and all possible interactions of LPS, GLY, and hour with block and steer within treatment combination (LPS × GLY) as random effects. Differences were considered significant when $P \leq 0.05$ and a tendency when $P \leq 0.10$.

RESULTS AND DISCUSSION

In Situ Ruminal Degradability

By design, soluble fraction A of the feed DM was not different ($P \geq 0.37$) among treatments (Table 2). A tendency for an LPS × GLY interaction ($P = 0.07$) occurred for both fraction B and C. The percentage of fraction B (representing the potentially degradable fraction) was not different between −GLY and +GLY for −LPS steers, but was lower when +LPS steers received +GLY vs. −GLY. This response was mirrored by the percentage of fraction C (representing the ruminally undegradable fraction) that also did not differ between −GLY and +GLY for −LPS steers, but increased when +LPS steers received +GLY vs. −GLY. Furthermore, the ruminal degradation rate of DM was not different when −LPS steers received +GLY or −GLY, but was lower when +LPS steers received +GLY vs. −GLY. Therefore, ruminal DM degradation was not negatively affected when −LPS steers were supplemented with glycerin, which is consistent with Wang et al. (2009) who observed a linear increase in ruminal DM degradation rate with glycerin supplementation up to 300 g/d. In our study, +GLY steers consumed 420 g/d of glycerin based on water intake measurements reported by Carey et al. (2017). In contrast, a decrease in ruminal DM degradation rates of +LPS steers when receiving +GLY is somewhat consistent with Ciriaco et al. (2015), who reported decreases in the in situ DM degradation rate of hay when steers were supplemented with 680 g/d of glycerin or more (even in the absence of a stress model). Therefore, glycerin supplementation may negatively affect DM degra-

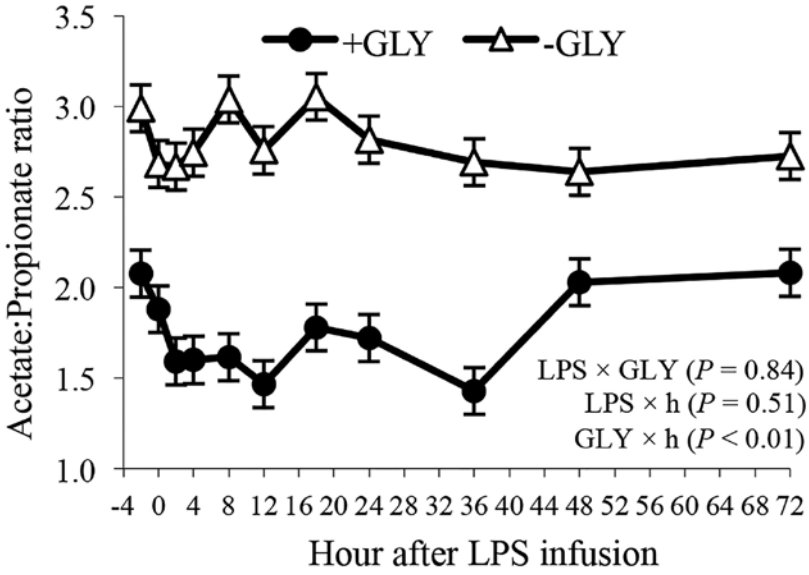

Figure 1. Ruminal pH and molar percentages of acetate, propionate, and butyrate in response to crude glycerin (GLY) supplementation in steers. Treatments (2 × 2 factorial) were GLY supplementation at 0 or 25 g/L of drinking water (−GLY vs. +GLY), and subcutaneous injection of lipopolysaccharide (LPS) at 0 or 3 µg/kg BW.

TABLE 3. Rumen fermentation characteristics in steers exposed to lipopolysaccharide (LPS) and supplemented with glycerin (GLY)

	Treatments[1]							
	-LPS		+LPS			*P*-value		
Item	-GLY	+GLY	-GLY	+GLY	SEM	LPS × GLY	LPS	GLY
pH	6.70	6.50	6.70	6.60	0.200	0.61	0.40	0.13
NH_3, m*M*	3.9	2.5	3.6	2.3	0.47	0.88	0.53	<0.01
Total VFA, m*M*	103.8	95.8	98.2	98.1	6.60	0.25	0.62	0.23
Individual VFA, mol/100 mol								
Acetate	60.1	50.8	60.2	49.3	1.60	0.61	0.65	<0.01
Propionate	22.1	32.2	22.4	28.9	1.65	0.28	0.37	<0.01
Butyrate	12.7	11.6	12.4	16.6	1.20	0.03	0.06	0.23
Isobutyrate	1.3	0.5	1.1	0.9	0.13	0.04	0.50	<0.01
Valerate	1.1	2.6	1.2	1.8	0.34	0.08	0.20	<0.01
Isovalerate	2.6	2.3	2.6	2.6	0.30	0.53	0.53	0.39
A:P[2]	2.8	1.7	2.8	1.8	0.20	0.84	0.77	<0.01

[1]Treatments were subcutaneous injection of 0 or 3 µg LPS per kg of BW (−LPS vs. +LPS; *Escherichia coli* O55:B5; Sigma Chem. Co., St. Louis, MO) dissolved in 2 mL of sterile saline, and energy supplementation of either 0 or 25 g/L crude glycerin (−GLY vs. +GLY) added to the drinking water.

[2]Acetate to propionate ratio.

dation in the rumen of steers when challenged with an endotoxin or when supplemented in large amounts.

Rumen Fermentation Characteristics

A GLY × h interaction ($P = 0.05$) occurred for ruminal pH; ruminal pH of steers was lower for +GLY than –GLY at 4 and 12 h, but not different among glycerin treatments for all other hours of sample collection (Fig. 1). Although total VFA concentrations and molar proportions of isovalerate were not different ($P \geq 0.23$) among treatments (Table 3), a GLY × h interaction ($P \leq 0.02$) occurred for molar percentages of acetate, propionate, and valerate (Fig. 1), and acetate to propionate (**A:P**) ratio (Fig. 2). Acetate in ruminal fluid of +GLY steers decreased from -2 to 4 h and was lower for +GLY than −GLY steers throughout the 72 h of collection (Fig. 1). In contrast, propionate in ruminal fluid of +GLY steers increased from -2 to 4 h and was greater for +GLY than −GLY steers for all hours of the 72-h collection. Thus, the A:P ratio (Fig. 2) in ruminal fluid of +GLY steers also decreased from -2 to 4 h and was lower for +GLY than −GLY steers at all hours of the 72-h collection period. Valerate in ruminal fluid of

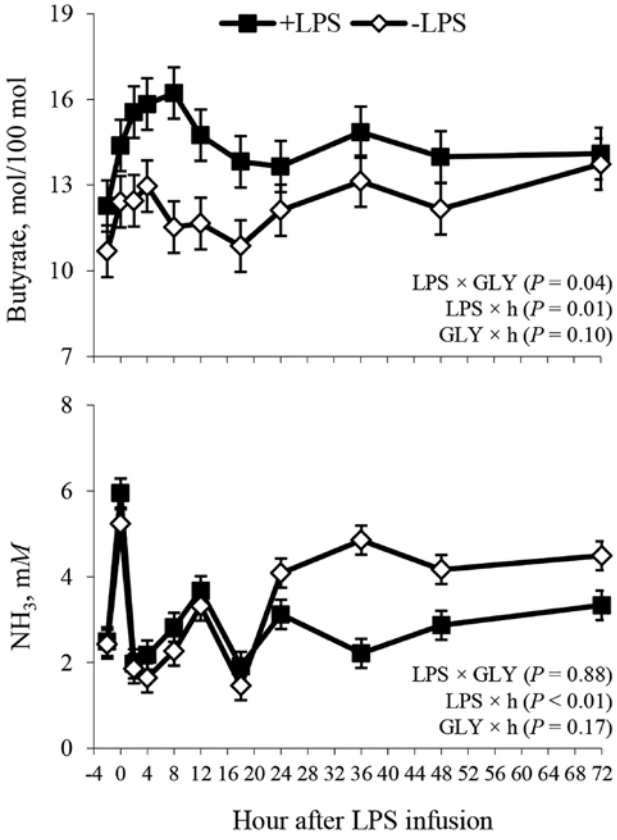

Figure 2. Ruminal acetate to propionate ratio in response to crude glycerin (GLY) supplementation in steers. Treatments (2 × 2 factorial) were GLY supplementation at 0 or 25 g/L of drinking water (−GLY vs. +GLY), and subcutaneous injection of lipopolysaccharide (LPS) at 0 or 3 µg/kg BW.

+GLY steers increased from -2 to 12 h and was greater for +GLY than –GLY through h 36 of collection, then decreased from 36 to 48 h and was not different between glycerin treatments at 48 and 72 h. Ruminal NH_3 concentrations were lower ($P < 0.01$) for steers receiving +GLY than −GLY (Table 3). These results indicate that glycerin supplementation shifts rumen fermentation in favor of propionate production and rumen NH_3 utilization. The observed decrease in A:P ratio in rumen fluid of glycerin-supplemented steers is similar to the results of Abo El-Nor et al. (2010) and Krueger et al. (2010), suggesting that glycerol is mainly fermented to propionate by rumen bacteria.

An LPS × h interaction ($P = 0.01$) occurred for molar percentages of butyrate (Fig. 3); butyrate increased from 0 to 8 h and was greater for +LPS vs. –LPS steers at 2, 4, 8 (peak), 12, and 18 h of ruminal fluid collection. An LPS × h interaction ($P < 0.01$) occurred for ruminal NH_3 concentrations; rumen NH_3 concentrations were not different for +LPS vs. –LPS steers from -2 to 18 h, but were lower for +LPS than –LPS steers at 24, 36, 48, and 72 h after LPS injection (Fig. 3). This decrease in ruminal NH_3 concentrations

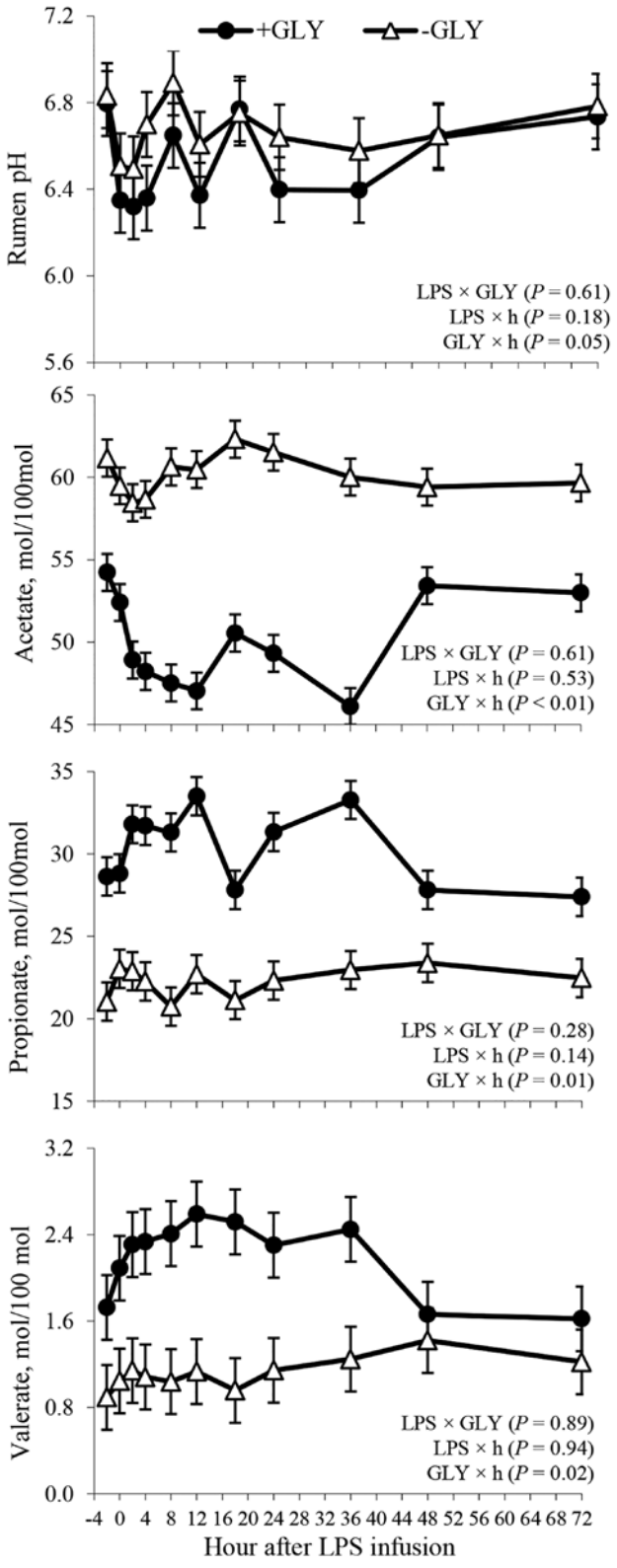

Figure 3. Ruminal molar percentages of butyrate, and NH3 concentrations in response to lipopolysaccharide (LPS) administration in steers. Treatments (2 × 2 factorial) were subcutaneous injection of LPS at 0 or 3 µg/kg BW (−LPS vs. +LPS), and crude glycerin supplementation at 0 or 25 g/L of drinking water.

may suggest that after stress, rumen microbes are either producing less NH_3 or utilizing more N to compensate for stress exposure.

Implications

These results imply that steers exposed to stress and supplemented with glycerin via drinking water have decreased ruminal nutrient degradability and rumen fermentation that favors glucogenic precursors. Glycerin supplementation did not alleviate the negative effects of stress on rumen function.

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Effect of feed efficiency and sexed semen on pregnancy rate and early embryonic mortality in beef heifers

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ABSTRACT: The objectives of this experiment were to examine the impact of 1) relative feed intake (RFI) or 2) semen type (SEXED vs conventional [CON]) on pregnancy rate in beef heifers. In addition, the effect of semen type on early embryonic mortality was examined. Crossbred heifers (n = 124) underwent RFI testing in a GrowSafe System for 90 d on an 80% hay: 20% concentrate diet. Heifers were classified as Efficient (< -0.5 SD), Average, or Inefficient (> + 0.5 SD). At 13 mo of age, heifers were weighed and reproductive tract scored (RTS). Heifers were blocked by BW and RTS and randomly assigned to be inseminated with SEXED or CON semen. All heifers were synchronized using the 14-d CIDR PG protocol with split-time AI. On d 0, heifers received a controlled internal drug release device (CIDR; Zoetis, Parsippy, NJ), and CIDR was removed on d 14. On d 30, heifers received PG (Lutalyse, 25 mg i.m.), an electronic mount detector (Accubreed; Estrotect, Denver, CO) and an estrus detection patch (Estrotect). At 66 h after PG, all heifers with activated patches were inseminated. Heifers with unactivated patches were inseminated 24 h later and given an injection of GnRH (Factrel, 100 μg i.m.). All heifers were bred to the same AI sire with either SexedUltra semen (4×10^6 cells/mL) or CON semen (20×10^6 cells/mL). Blood samples (5 mL) for Pregnancy-Specific Protein B (PSPB) analysis (BioPryn; BioTracking, Moscow, ID) were obtained on d 25 and d 30 after AI. Pregnancy rate (PR) and fetal age were determined via ultrasound at d 41, d 62 and d 120 after AI. Effect of semen type on PR was analyzed by Proc Freq procedures of SAS. Effect of RFI, BW or RTS on PR, and effect of semen type on PSPB concentrations were analyzed by Proc GLMMIX of SAS. Residual feed intake classification did not affect PR ($P > 0.27$). AI PR (d 62) was similar ($P = 0.59$) for SEXED (60.3%) or CON (63.9%). Comparison of PR as indicated by PSPB on d 30 with AI PR at d 41 indicated an embryo loss of 6% and 5% for CON and SEXED, respectively. Neither RTS nor BW influenced PR ($P > 0.22$). In conclusion, RFI does not affect reproduction in beef heifers. Furthermore, in this study, PR and early embryonic mortality were similar in heifers inseminated with sexed or conventional semen.

Key words: beef heifers, reproduction, residual feed intake, sexed semen
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Effects of supplementing CRINA ruminant blend essential oil to Suffolk x Hampshire feeder lambs on growth, intake, feed efficiency, and carcass traits

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ABSTRACT: Objectives of this trial were to determine effects of supplementing CRINA ruminant blend essential oil feed additive on performance measures of growing, Suffolk x Hampshire lambs. A total of 16, fall born lambs (100 ± 5.3 d of age; ewes n = 6; wethers n = 10) were randomly sorted into three treatments groups and stratified by BW and sex. All lambs were fed a base finishing ration of 65% commercial grain mix and 35% alfalfa sweeps (CP:18.18% TDN: 67.91% NDF: 24.64% on a DM basis). The ration was balanced to meet NRC requirements for finishing lambs. Treatments included 1) control (CON; n = 5; initial BW 30.12 ± 8.95 kg), 2) high essential oil (HEO; n = 5; initial BW 30.12 ± 7.17 kg), and 3) low essential oil (LEO; n = 6; initial BW 30.54 ± 5.66 kg). The LEO and HEO treatments had 75 and 150 mg of the EO blend added to the control ration, respectively. The CON group received no CRINA supplement, LEO and HEO group received essential oil supplement at a rate of 75 and 150 mg per hd per d, respectively. Lambs were fed individually in (76.2 cm X 154.4 cm) stalls for a minimum of 45 mins and refusals were weighed back to determine lamb intake. Rations were calculated based on individual animal intake measures and adjusted accordingly. Animals were given *ad libitum* access to water and generic sheep mineral. Body weight data were collected on d 0, 28, and 56 of the trial and used to determine individual ADG, feed to gain ratio, feed intake, and total gain. Feed to gain ratio, feed intake, BW and carcass yield traits were not different ($P > 0.05$) among treatment groups. Respectively, ADG and total gain were different ($P = 0.01$) between HEO (0.45 ± 0.32 kg; 25.03 ± 3.20 kg) and both the LEO (0.36 ± 0.14 kg; 19.95 17.61 ± 4.02 kg) and CON (0.31 ± 0.32; 17.61 ± 2.02) groups. Although there have been implications that supplementing livestock species with essential oils can have several benefits to growth performance, more research is needed to determine whether it is a cost-effective feed additive for producers.

Key words: essential oil, feed efficiency, supplementation
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Comparison of two estrus synchronization protocols utilized with natural service in young beef cows on reproductive performance and profitability

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ABSTRACT: Eighty-eight crossbred Angus-based cows (2-3 yr old) were utilized to determine the effectiveness of 2 estrus synchronization protocols on reproductive efficiency and economic viability when coupled with natural service. Cows were stratified by d post calving and randomly assigned 1 of 2 synchronization protocols: 1) Select Synch + CIDR (CIDR) or 2) a single injection of prostaglandin administered 4.5 d after exposure to bulls (PG). Heat detection aids were placed on cows at d 0, corresponding with d of CIDR removal. Bulls were placed with cows at a 1:18 bull:cow ratio for a total of 60 d. Estrus was monitored twice d for 10 d to identify animals responding to synchronization protocols. Forty-five d after bull exposure and 45 d after bull serum samples were collected on each cow to diagnose pregnancy. Estrus response was similar (P = 0.66) over the first 10 d of the breeding season for CIDR (42.2 ± 7.4%) and PG (37.5 ± 7.8%) cows. Similarly, early pregnancy rate (55.8 vs 66.7 ± 7.9%) and final pregnancy rate were similar ($P \geq$ 0.32) for CIDR compared with PG cows, respectively. Cows treated with the CIDR protocol calved earlier (P = 0.005) compared with PG (55.6 vs. 60.8 ± 1.2 d, respectively). However, the proportion of cows calving with the first 21 d of the calving season was similar (P = 1.0) among treatments. Cost of synchronization between the two systems was $17.21/cow less for the PG treated compared to CIDR treated cows. Differences in cost can be associated with cost of CIDR, as well as, additional labor required for the CIDR protocol. Reproductive efficiency of young cows was similar when comparing CIDR and PG based synchronization protocols. However, utilization of the PG protocol would reduce synchronization costs to producers without impacting reproductive performance.

Key words: beef cow, CIDR, estrus synchronization, prostaglandin

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Evaluating the use of BMR (Brown Midrib) corn as an acceptable forage source for grazing cattle

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ABSTRACT: Corn can be a valuable forage substitute for many areas, including Montana. Brown Midrib (BMR) corn is a hybrid corn, with reduced lignin and improved digestibility, allowing for greater dry matter and nutrient intake. While BMR corn has been widely used as an ensiled product, little information is available regarding its use as a grazing source. Our objective was to evaluate the utility of three BMR corn varieties as a forage grazing source. Our hypothesis was that the corn varieties would be adequate for grazing livestock. The study was a randomized complete block design, with three blocks and three replications of three BMR varieties within each block. Corn was planted on June 10, 2015 and sampled on August 24, 2015. A 0.3 m×0.3 m sample was hand-harvested from each plot. Samples were weighed and dried for dry matter and nutrient analysis. Samples were analyzed for nitrates, neutral detergent fiber (NDF) and acid detergent fiber (ADF). Crude protein (CP) and digestibility will follow. NDF values were significantly impacted by both replication (P = 0.0002) and variety (P = 0.0079), with replication 1 having the highest NDF and replication 3 having the lowest. Variety 2 was significantly lower ($P < 0.05$) than varieties 1 and 3. ADF was significantly impacted by replication (P = 0.0001), with replication 1 having the highest ADF, and replication 3 having the lowest. Nitrate levels were not affected by replication ($P = 0.1221$) or variety (P = 0.1950), and all were under toxic levels. This information indicates that corn can be a suitable source of forage for grazing livestock in Montana.

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Volatile fatty acids concentrations of preweaned beef calves from birth to weaning

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ABSTRACT: A study was conducted with the objective to determine if calves raised on native rangelands in New Mexico (NMANG; n = 10) differ in ruminal VFA levels from calves raised in Nebraska on predominately smooth bromegrass (*Bromus inermis*) pastures (NEANG; n = 10). Our hypothesis was that pastures with diverse plant communities and differing diet quality will result in an alteration of ruminal VFA concentration as calves' age and diet quality changes with season. Both locations utilized Black Angus calves and ruminal samples were collected on d 7, 63, 91, 119, 147, and 175 ± 5 of age via oral lavage. The pastures at each location differed by plant species and diet quality. Ruminal VFA concentration was determined by gas chromatography. The effect of location, calf age and, location X calf age was on ruminal VFA production was evaluated. Total VFA and butyrate differed for location X calf age ($P < 0.001$). Acetate and propionate differed by location ($P < 0.01$). Propionate was 13.6% greater in calves raised in New Mexico while Nebraska raised calves had 5.4% greater acetate. The acetate:propionate ratio tended to increase in Nebraska calves vs. New Mexico calves ($P = 0.08$). These data support our hypothesis that diet quality and plant species impact ruminal VFA levels in calves. Ruminal VFA serve a primary source of energy for the host animal and are indicative of the development of the ruminal microbial populations.

Key words: ruminal VFA, Angus, Preweaned calf
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Post-weaning growth performance and feed efficiency of commercial and half-blood Lowline-Angus heifers

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ABSTRACT: Drought conditions in much of the Western United States have caused an increase in feed costs for beef production systems; therefore, there is an increased interest in the performance of smaller biological-type cattle. Lowline-Angus were developed to reach a smaller slaughter weight (i.e. 400 kg) in the 1950s. Utilizing half-blood Lowline-Angus (i.e., half-blood) heifers in commercial cow herds may allow ranchers to maintain conventional production methods, while accessing a niche or direct market. Minimal information is available about the performance of Lowline-influenced cattle. Objectives of this study were to measure growth performance and feed efficiency of commercial (n = 19) and half-blood Lowline-Angus (n=20) heifers during the post-weaning and development phases. A total of 39 spring-born heifers were delivered to the CSU, Chico cattle feeding facility 45 d post-weaning and randomly assigned to 3, 7 x 18 m pens equipped with GrowSafe feed intake system for 21 d adaption period. Commercial (n = 19) and half-blood Lowline-Angus (n = 20) heifers were fed a forage-based developing ration (CP: 14%, TDN: 54%, NDF: 39.7%, DM basis) for 72 d and allowed *ad libitum* access to feed and water. The trial concluded after 72 days and BW collected on d 0, 1, 35, 71 and 72. Data were analyzed as a randomized block design (block = pen). Breed x pen effect was not significant ($P > 0.05$). Initial weight (311.92 ± 2.81 kg vs. 280.53 ± 3.66 kg) and metabolic mid-weight (67.00 ± 0.67 kg. vs. 59.70 ± 0.61 kg) were different ($P < 0.0001$) among commercial and half-blood heifers. Residual feed intake did not differ among breed types ($P > 0.05$); however, ADG and dry matter intake were different ($P < 0.001$) for commercial and half-blood heifers (ADG; 1.18 ± 0.13 kg vs. 0.65 ± 0.06 kg; DMI; 12.66 ± 0.41 kg vs. 10.23 ± 0.48 kg). Differences in growth measures may reflect biological type; however, RFI results suggest that efficiency differences are absent among commercial and half-blood Lowline-Angus heifers. Cumulatively, results of this study indicate that half-blood Lowline-Angus heifers may have the ability to perform as efficiently as commercial contemporaries. However, further research should be conducted to better understand of the long-term effect of RFI on economically important traits in beef production.

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Impact of ensiling sugar beets with or without a mold inhibitor on internal temperature, nutrient composition, digestibility, and pH

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ABSTRACT: Non-harvested sugar beets represent an abundant and yet underutilized feedstuff for livestock producers in Montana and Northern plains region. The moisture content of sugar beets may create opportunities for improving storage and ensiling options. The objective of this project was to determine if sugar beets could be ensiled with hay and soybean meal with or without a liquid mold inhibitor (Ultra CURB®; Kemin Industries, Inc.) and the impact of ensiling sugar beets on internal temperature, nutrient composition, and pH. A 3 x 2 factorial experiment where hay (control; H) or sugar beets mixed with either hay (SBH) or soybean meal (SB) were ensiled at a rate of 50:50 (as fed) without the mold inhibitor. The mold inhibitor was included to create three additional treatments: hay (HT) sugar beets:soybean meal (SBT), and sugar beets:hay (SBHT).

Water was added to each mini-silo to achieve an optimum dry matter (35%). Temperature sensors were included in each replicate to monitor internal temperature. Data were analyzed using the Mixed procedure of SAS. Ash content (P = 0.001) was greatest in H, with SBH being intermediate, and SB being the least. Ash content of the silages was not altered by the mold inhibitor (P = 0.20) or the 90-d ensiling process (P = 0.64). Nitrogen concentrations (P < 0.001) were greatest for the SB silage, with SBH being intermediate, and H being the least. The 90-d ensiling process did not alter (P = 0.35) N concentrations compared with d 0 samples. The HT silage tended to have greater (P = 0.07) N content than the H silage, and the SBT and SBHT silages had greater (P ≤ 0.01) N content than the SB and SBH silages. Fiber content (NDF and ADF) was greater (P = 0.008 and P = 0.002, respectively) in the H and SBH treatments compared with the HT and SBHT silages, respectively. Density was greater (P < 0.001) in the SB silage compared with the SBH and H silages. Treating the silages with the mold inhibitor did not alter (P = 0.33) silage density. Preliminary data suggest, sugar beets may be ensiled with hay or soybean meal and with or without a mold inhibitor without negatively impacting nutrient quality. Effects of aerobic stability, in situ digestibility, and duration of ensiling are concurrently being evaluated and results are forthcoming.

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Maternal nutrition and day of gestation influence the expression of neutral and acidic amino acid transporters and their substrate concentrations in bovine utero-placental tissues and fluids from d 16 to 50 of gestation

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ABSTRACT: We hypothesized that maternal nutrition and day of gestation would influence the relative gene expression of neutral and acidic AA transporters *SLC1A1, SLC38A7,* and *SLC7A5*, in heifer utero-placental tissues, as well as the concentrations of their substrates in heifer utero-placental fluids. Angus-cross heifers (n = 49) were synchronized, bred via AI, assigned to nutritional treatment (CON = 100% of requirements for 0.45 kg/d gain and RES = 60% of CON) and ovariohysterectomized on d 16, 34, or 50 of gestation (n = 6 to 9/d); non-bred (NB) controls were ovariohysterectomized on d 16 of the estrous cycle (n = 6). The resulting arrangement was a 2 × 3 factorial + 1. Caruncle (CAR), inter-caruncular endometrium (ICAR), and fetal membranes (FM), histotroph, allantoic, and amniotic fluid were obtained from the uterine horn containing the conceptus. Relative expression of *SLC1A1*, *SLC38A7*, and *SLC7A5* was determined for each tissue using NB-CAR and NB-ICAR tissue as the baseline. For FM, NB endometrium served as the baseline. Expression of *SLC1A1* and *SLC7A5* were not influenced by a day × treatment interaction in any tissues ($P > 0.05$); additionally, *SLC7A5* was not affected by day or treatment. In both CAR and ICAR, *SLC1A1* was greater ($P < 0.01$) on d 16 compared with d 34 and 50. In ICAR, *SLC38A7* was influenced by a day × treatment interaction, in which d 16 RES was greater ($P < 0.01$) than all other days and treatments. Glutamine concentration was influenced by a day × treatment interaction in amniotic fluid, being greater ($P = 0.04$) on d 34 RES compared with all other days and treatments. Valine concentration in allantoic fluid was influenced by a day × treatment interaction ($P = 0.05$), such that d 50 CON was greater than all other days and treatments. Aspartate concentration in histotroph was greater ($P = 0.02$) in RES compared with CON heifers. In allantoic fluid, aspartate concentration was greater ($P = 0.03$) in CON compared with RES. In allantoic and amniotic fluids, leucine concentration was greater ($P \leq 0.01$) on d 50 compared with d 34; additionally, leucine was greater ($P = 0.03$) in histotroph on d 50 compared with d 16 and 34. These data support our hypothesis that maternal nutrition and day of gestation influence the expression of neutral and acidic AA transporters and the concentrations of neutral and acidic AA in bovine utero-placental tissues and fluids.

Key words: aspartate, glutamine, leucine, valine
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Supplementation of crude glycerin via drinking water alters feed intake of sheep

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ABSTRACT: Glycerin, a biodiesel byproduct, has been used as an energy supplement for livestock and has been reported to suppress feed intake at high concentrations when included in ruminant diets. Decreased feed intake associated with stressed, morbid livestock makes it difficult to meet the animal's nutrient requirements. Glycerin supplementation in drinking water offers an alternative source of energy without altering energy-density of the diet or increasing DMI. We hypothesized that supplementing yearling ewes with various concentrations of crude glycerin in their drinking water would affect DMI, water intake, and serum glucose concentrations. The purpose of this experiment was to determine optimal concentration of glycerin in drinking water for ewes by evaluating the effects of crude glycerin supplementation at 0, 25, 50, and 75 g/L on DMI, water intake and serum glucose concentrations. Four Rambouillet yearling ewes (66 ± 2.7 kg BW) were used in a 4 × 4 Latin square. Each period consisted of 5-d, 3-d of adaptation to treatments and 2-d of collection. All variables were analyzed using the GLM procedure of SAS and the statistical model included the effect of sheep, treatment, day, and period. Linear and quadratic contrasts were tested for treatments. The crude glycerin contained 85% glycerol and < 100 ppm methanol, which is generally regarded as safe for livestock consumption by the US Food and Drug Administration. Sheep were housed individually with visual contact of each other and were fed (ad libitum) a corn silage-based diet (1.74 Mcal/kg NEm, 8.0% CP, 0.47% Ca, and 0.26% P on a DM basis) once daily. Orts were collected daily, and sheep were fed 10% more than the previous day's intake. Water intake was measured by weight on each day of the collection period prior to refreshing each sheep's water treatment to an allocated 20 L/d. Jugular venous blood samples (10 mL) were collected on d 5 of each period. Dry matter intake (1.19, 1.29, 1.12, and 0.87 ± 0.086 kg/d for 0, 25, 50 and 75 g/L) had a quadratic effect (P = 0.05), with DMI being lower for 75 g/L than 0, 25 and 50 g/L. There were no effects of treatment on water intake ($P \geq 0.36$) or serum glucose ($P \geq 0.51$). These results imply that sheep may consume glycerin supplementation up to 50 g/L in water without negatively affecting DMI of a corn silage-based diet.

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Lipid droplets (LD) in the corpora lutea (CL) during early pregnancy in cows fed different planes of nutrition

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ABSTRACT: The CL is the primary source of the gestational-supporting hormone progesterone (P4). Lipid droplets are cellular organelles serving as energy reservoirs, providing precursors for steroid and other hormones, and mediating physiological and pathological conditions. We hypothesized that plane of nutrition and pregnancy stage would alter LD accumulation in luteal tissues and serum steroid hormone concentrations. The objective was to determine the effects of dietary restriction (RES) on luteal LD accumulation, and serum P4 and estradiol-17β (E2) concentrations. Commercial Angus crossbred heifers (n = 9/treatment) were fed a total mixed ration supplemented with dried distillers grains with solubles, and ad libitum water access. On the day of breeding, heifers were randomly assigned to dietary treatments in a 2 × 3 factorial arrangement of nutritional plane × stage of gestation. One half were assigned to a control diet (CON; 100% of requirements) and the remaining half to a RES diet (60% of CON). On d 16, 34, and 50 of pregnancy, serum samples were collected for P4 and E2 determination, ovariohysterectomy was performed, and CL were dissected for assessment of LD accumulation. A portion of each CL was frozen in OCT, sectioned using a cryostat, formalin-fixed, and stained with BODIPY, a marker of LD. Images of luteal tissues were generated using microscopy followed by image analysis to determine percentage of tissue area occupied by LD. The luteal area occupied by LD was less ($P < 0.03$) on d 34 and 50 than on d 16 (0.8 ± 0.1 and 0.9 ± 0.2 vs. 1.5 ± 0.3%). Interactions ($P = 0.1$) between day of pregnancy and plane of nutrition demonstrated that on d 16, LD accumulation was less in RES compared to CON (1.0 ± 0.2 vs. 1.9 ± 0.4%). Serum P4 concentration was less ($P < 0.02$) in RES than CON on d 34 and 50 (7.1 ± 0.8 vs. 3.7 ± 0.6, and 6.9 ± 0.9 vs. 3.3 ± 0.6 ng/mL). Plane of nutrition and pregnancy stage did not affect serum E2 concentration. Thus, during early pregnancy, LD accumulation in luteal tissue and serum P4 were affected by plane of nutrition. These data emphasize importance of the diet for maintaining normal reproductive functions including luteal LD expression and P4 secretion. Understanding mechanisms of steroid production and the role of LD in CL tissue may help to establish strategies to improve pregnancy rates in livestock.

Key words: corpora lutea, heifer, lipid droplets, pregnancy, nutrition
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Reflections on becoming an animal science generalist

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ABSTRACT: Most academic animal scientists embark on their professional careers with reasonably narrow, discipline-specific training. Some build their careers in that narrow area, whereas others develop a broader approach to their scholarship, becoming, for lack of a better term, animal science generalists. Generalists in all fields of science can be important contributors to multi- and transdisciplinary teams working to solve larger, more complex problems (grand challenges). Indeed, because of their breadth of understanding across disciplines, they often make effective team leaders. In my own career, which strayed far on occasion from the fundamentals of ruminant digestive physiology and metabolism in which I was formally trained, numerous factors led to my development as an animal science generalist. Among these factors were educational processes before disciplinary training, mentoring, opportunities of time and place, and collaborations.

Key words: scientific generalist, disciplinary training, grand challenges
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INTRODUCTION

When you are young, it seems easy to know exactly where you are going and what you plan to do. You know you want to be something in particular – in my case, a ruminant nutritionist – and you have a graduate program to finish, a job to start, and a clear-cut research agenda that is honed in on a specific little corner of the discipline (typically driven by your dissertation research). If you are fortunate, it often starts out that way, and at least for a little while, you get to do what you have always dreamed you would be able to do. But the challenge everyone faces is keeping it that way – keeping that laser focus on the particular little corner of your discipline that you cherish so much becomes an increasing challenge. Some people are able to maintain that focus and to spend their entire career in that corner, often doing remarkable things and discovering critical new knowledge that enlightens their favorite corner and the broader discipline as well. It is not necessarily a bad thing to stay in that corner. Others of us only stay in that corner for a while and perhaps return to it now and then, but by our very nature, we get distracted and venture into new territory. Maybe we get distracted in the quest for research funding, or sidetracked by that really sharp graduate student whose inquisitive nature and great ideas pull us into another line of research, maybe we just read an article that piques our interest and tempts us to travel down another path, or maybe we are just by nature "generalists."

In my own case, it was probably a combination of all the things listed above, and a few I might not even recognize as having an influence, that led me down my particular career path. Although a ruminant nutritionist by training, I feel fortunate that my career has expanded well beyond the boundaries of the rumen. I have become, for lack of a better term, an "animal science generalist." In this paper, I plan to share some of my favorite memories of this journey down the road to becoming a generalist. I hope by so doing, I might encourage young animal scientists to see the value in broad thinking and flexibility in setting the direction of their careers. As my career has been spent entirely in an academic setting, my reflections will be from that perspective.

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REFLECTIONS

On the Value of Being a Scientific Generalist

"Grand challenges," "wicked problems," and other such phrases that describe complex scientific and social problems are common terms these days. These are problems that are not "owned" by any single discipline. The words multidisciplinary and more recently, transdisciplinary, are used extensively by the scientific community in reference to the approaches needed to address grand challenges. Although the grand challenge concept is often applied on a global level across many scientific disciplines, it also has been adopted by narrower multidisciplinary organizations. For example, the American Society of Animal Science (ASAS) has developed a series of grand challenges: (https://www.asas.org/docs/default-source/public-policy/gc_pages_web2015.pdf?sfvrsn=2).

When it comes to graduate education, however, for the most part, we still live in a disciplinary world. We want our students to master disciplinary knowledge, to demonstrate their mastery by written and oral testing, and to conduct research to discover new knowledge in the discipline. Then we tell students that they need to be prepared to serve on multi- or transdisciplinary teams to solve the grand challenges that face their broader disciplines. So if all we need to do to solve the world's problems is put a group of scientists trained in specific disciplines in a room and let them hammer out the answers to the problems that face the world (or at least our general disciplinary part of the world), is there value to the scientific generalist? Specific to animal science, is their value to being a generalist? Or should everyone focus on their discipline – ruminant and non-ruminant nutrition, reproductive physiology, animal breeding, and so on?

Perhaps not surprisingly, many have argued over the years that there is a need for the generalist in science. A Google search for something like "Value of Scientific Generalists" will yield a large number of results for articles and books on the subject. And it is not a new argument. One of the most eloquent papers in favor of training scientific generalists is the work of Bode et al. (1949). This group of eminent scientists (primarily statisticians), said it this way:

"Scientific and technological advances have made the world we live in complex and hard to understand. We have today large scale division of labor, complex and indirect methods of production and distribution, large communities and large areas held together by common channels of transport and communication, and operation with small margins of safety, requiring close and delicate control. All these complex and delicate activities produce scientific and technological problems of great difficulty….We need a simpler, more unified approach to scientific problems, we need men who can practice science – not a particular science – in a word, we need scientific generalists. Research teams, aggregations of men of diverse skills working on the various aspects of single problems, have been widely used and have accomplished much. Their use is certain to continue and expand. But the research team must have a leader to unify the group, whether he be director, coordinator, or advisor. This leader must work as a scientific generalist, and we feel he would function better if trained with this in mind."

If one was unaware of the date of publication and overlooked the gender-specific references we would be unlikely to use in current writing, one might think that Bode and colleagues were making a case for teams of scientists to solve all those grand challenges and wicked problems we face today. Their case is a solid one – the generalist is a natural multi- or transdisciplinary team leader. He/she is the person who has the breadth of understanding of several disciplines (or sub-disciplines within a discipline like animal science) to provide effective leadership and to potentially understand the broader picture that allows the specific disciplines to be brought together in problem-solving activities.

So, is there value to being a scientific generalist? The answer is arguably "yes." Is it the right path for everyone? The answer is most likely "no." Put too many generalists on a problem-solving team, and the likelihood of solving problems would probably diminish. As with most undertakings in life, it takes a balanced approach for things to work properly, and a good generalist leading a strong team of well-trained specialists can do some remarkable things.

On Becoming an Animal Science Generalist

If indeed I am the animal science generalist that I claim to be, how did I get there? Looking back, I think there were many contributing factors, but the keys ones I have identified and will discuss briefly here are education, mentoring, time and place, and collaborators.

Education. Becoming who we are as scientists starts early – long before graduate school, my approach to the world was shaped by parents who valued education (and encouraged me to read about all kinds of things) and by a series of elementary and secondary educators who managed to impart a sense of importance to the idea of understanding the world around me and providing the tools (reading, writing, and mathematics) to start deciphering it. At the university level, I started out like most animal scientists do, finishing a B.S. degree with a focus on general animal

science – the word "general" being important here. We do not train specialists at the baccalaureate level – we train generalists. In addition to that general training, however, I was fortunate to work during most of my college years in the animal nutrition laboratory, and at New Mexico State University (NMSU), that meant ruminant nutrition.

Thus, when it came time to think about graduate study, I knew that ruminant nutrition was exactly the right path for me. And I took the deep dive into the discipline, gobbling up all the discipline-specific knowledge the ruminant nutrition faculty at Oklahoma State University (OSU) could dish out and mastering the nuances of rumen sampling, measurements of digesta kinetics, and site of digestion studies that would serve me well over the next decade as a young faculty member. But I also discovered something else in graduate school that was, at least in my case, a vital part on the road to a generalist view – statistics. Statistics seemed like such a wonderful tool that I chose it as a minor rather than Biochemistry like most of my fellow graduate students – and it has proved to be a terrific asset throughout my entire career.

Education does not stop when you take your first job and become a faculty member (or a consulting nutritionist, or a technical services representative, etc.). What I learned over the next several years in preparing lecture notes for classes, particularly graduate classes, was probably far more than I had learned in graduate school. Essentially, I learned the important lesson that there is always much to learn. Some faculty colleagues might find it hard to believe, but even as a university administrator, I am still learning that lesson today!

Mentoring. I am convinced that mentoring is a key factor in determining who we become as scientists. Throughout our careers, we experience mentoring in two ways – as the one being mentored and as the mentor. As an undergraduate worker in the animal nutrition laboratory at NMSU, I was fortunate to have G. Stanley Smith as my academic advisor and mentor. "Stan" let me explore ruminant nutrition through undergraduate research and helped me ultimately make the choice of graduate school over a veterinary education. Stan was a classically trained scientist who was an incredible storehouse of knowledge – a scientific generalist without doubt. At OSU, I was fortunate to be trained by similarly accomplished scientists – Fred Owens, Don Wagner, and Gerald Horn being key among the ruminant nutrition faculty members. Although gifted scientists in their specific discipline areas, they were also very broadly interested in animal production and how nutrition and physiology related to it. And after I returned to NMSU to join the faculty there, Joe Wallace introduced me to the world of animal production and nutrition on native rangelands.

Serving as a mentor of graduate and post-doctoral students was, however, very likely the defining activity on my road to becoming an animal science generalist. At the risk of excluding and offending some of my 62 M.S. and Ph.D. advisees, a few examples might be worthwhile. Mike Hubbert was one of my first graduate students. Mike was interested in everything, and I think his own inclinations to be a generalist were instrumental in heading me down the generalist path. Because of research at the Clayton Livestock Research Center (CLRC) that I was involved with, which I will mention again in a subsequent section, Mike and I developed and interest in animal health and feed intake that has stayed with us for life – and we also developed a lifelong friendship.

Ted McCollum was another of my very early graduate students. With a B.S. in Biology from Baylor University, he did not have a typical animal science background, but Ted was from a ranching family in Fort Sumner, NM, so he had a solid understanding of the cattle business and grazing livestock. After completing a standard "take lots of rumen samples and measure everything you can" M.S. degree, Ted approached me about conducting his Ph.D. work at Fort Stanton, NM, to look at seasonal changes in forage intake, digesta kinetics, and ruminal fermentation. Although I had interest in working with harvested forages in pen-based models, I had not given any serious thought to taking those techniques to the "field." But Ted convinced, me, and we decided to venture forth. To do so, however, we needed to conduct a test run on the use of ytterbium-labeled forage to measure rate of passage, which we did in the context of a very simple experiment with ruminally cannulated animals fed prairie hay with or without protein supplement. That work (McCollum and Galyean, 1985a), which was functionally a training exercise, is among the most highly cited papers that my students have produced. Ted's ultimate Ph.D. work (McCollum and Galyean, 1985b; McCollum et al., 1985) was an important contribution, demonstrating that intensive measurements of digestive physiology could be made in ruminants grazing rangelands, which led to several other studies at NMSU and other universities in the years that followed.

Two other graduate students and one post-doctoral student should be noted as influencers on my road to a generalist approach. Paul Defoor completed his M.S. degree with me at West Texas A&M University (WTAMU) and then moved to Texas Tech University (TTU) to work on a Ph.D. program. Paul, a serious and hard-working young man, had a strong interest in the feedlot industry. He became intrigued with the import-

ance of roughage in feedlot diets, and he convinced me that we needed to work on the very practical issue of developing a system for "exchanging" roughage sources in feedlot diets. His work led to the conclusion that roughage sources can generally be exchanged on an NDF basis (although there is some effect of the physical effectiveness of the NDF), with a summary and extension of that work ultimately being presented as an invited paper at the ASAS meetings (Galyean and Defoor, 2003). The concept has been confirmed in several studies since then. Joe McMeniman, came to work with me after completing his undergraduate program at the University of Queensland in Australia. After wrapping up a feedlot study for his M.S. thesis, Joe wanted to work on prediction of feed intake by feedlot cattle. Given my previous interest in prediction of intake related to beef cattle nutrient requirements, I was certainly supportive. Joe's work led to extensive collaborations with commercial cattle feeding companies, and we were able to use their large data sets to develop new equations (McMeniman et al., 2010) that eventually became part of the 8th revised edition of the Nutrient Requirements of Beef Cattle (NASEM, 2016). Joe's influence on my generalist view of animal science was significant because it expanded my interest in meta-analytical methods and marked the start of collaborations with Dr. Luis Tedeschi noted below. The post-doctoral student who had a great influence on my thinking and approach to animal science was Judson Vasconcelos. Judson completed his Ph.D. at Texas A&M, but his M.S. program was at WTAMU, during which time he took a couple of graduate nutrition courses with me. I hired Judson as a post-doctoral student with the primary responsibility of cleaning up a backlog of data and manuscripts that had accumulated during my service as Editor-in-Chief of the *Journal of Animal Science* (*JAS*). Justin was exceptionally good at the job, and the backlog was cleared in no time. Then he started doing what post-doctoral students should do – formulating new ideas for research. One of his ideas, which frankly I was somewhat skeptical of at the time, was to expand a small survey of consulting nutritionists we had done a few years earlier and publish it in *JAS*. He convinced me, however, and we were able to get 29 consulting nutritionists from across the country to share their "trade secrets" – nutritional and management practices. The paper that came from that work (Vasconcelos and Galyean, 2007) has been highly cited and has served as a benchmark for practices in the industry. It is a long way from fundamental ruminant nutrition, and something I would never have thought of doing as a young faculty member.

Again, I could mention many more students who influenced my career path – all of them really, including some that were not my advisees. Students bring a unique perspective that influences the direction of one's research program, and in my case they certainly made that direction more general than specific.

Time and Place. The science that I have been involved with over the years also has been influenced by circumstances and the opportunities those circumstances present. After finishing my Ph.D. program at OSU focused on site of digestion as influenced by grain processing, the move back to NMSU to join the faculty in 1977 forced me to think about how my research skills could fit in a department that had very little activity focused on feedlot nutrition. Fundamental training that I have received at OSU about forage nutrition suddenly became more important, and my focus was on how to make my repertoire of research techniques fit in the context of a department in which grazing livestock was the dominant force. With the help of good students already mentioned and faculty mentors like Joe Wallace, that is exactly what happened, and over the next decade that is where my efforts were placed.

About the same time I moved to NMSU, however, the department embarked on a new venture – the CLRC. Headed by Dr. Glen Lofgreen, who had retired from the University of California System, the CLRC was focused on the health and management of newly received cattle. With the CLRC came some federal animal health money, and as the new kid on the block, I was tasked with figuring out how to develop some research activity in that area that would complement Dr. Lofgreen's work and be conducted at the CLRC. Being in that place at that time, led to the development of two lifelong interests: animal health (particularly newly received cattle) and animal management. As a result of those interests, I stepped out of the realm of ruminant nutrition and into the worlds of nutrition–immunity interactions (which opened new doors in mineral and vitamin nutrition), feed intake prediction and management, and collaborations with colleagues in the cattle feeding industry that have endured over the years. Also as a result of that work, I ultimately moved to Clayton to be the CLRC superintendent from 1990 to 1996.

For the last 19 years, I have been a member of the TTU faculty, and opportunities of time and place continue to present themselves. Located near the epicenter of cattle feeding in the U.S., TTU presents occasions to collaborate with the various industry groups that would be unlikely to happen at any other place. Examples include building the North American TBA Implant Database, a joint effort with Merck Animal Health, and participating in the Consortium for Cattle Feeding and Environmental Sciences (a collaborative effort between universities, the USDA, and industry groups). Collaborations with TTU faculty colleagues

(David Wester for one) also have enhanced my statistical abilities far beyond the meager beginnings provided by the statistical methods I learned as a student at OSU. Overall, what I have surmised is that once a person becomes a generalist, they start looking for opportunities of time and place to expand their generalist knowledge.

Collaborations. The final "force" driving me towards a generalist approach has been collaborations. I have already mentioned Mike Hubbert, who has been a lifelong collaborator – and probably the primary person responsible for the intake prediction equations in the NRC (1996) beef nutrient requirements publication. Others who come to mind are Andy Cole, one-time graduate school roommate, lifelong friend, and occasional collaborator. Indeed now that he is retired, Andy and I along with Luis Tedeschi and Mark Branine (a former Ph.D. student and true gem of a person who happened to be in possession of some of Dr. Lofgreen's original notes and data on the California Net Energy System) recently worked on an analysis of literature data to re-evaluate the relationship between ME and DE in beef cattle diets (Galyean et al., 2016). This work was an offshoot of our service on the National Research Council (now simply National Academies of Sciences, Engineering, and Medicine - NASEM) Committee on Beef Cattle Nutrition. I was privileged to be the chair of that committee, having served previously as a member the committee that produced the NRC (1996) publication. If you want to become an animal science generalist (or just a better scientist in general), do not turn down the opportunity to serve on a NASEM committee – it is a remarkable means of expanding one's knowledge of animal production, ranging from utilization of all the classes of nutrients to animal growth and reproduction, couched in an overall framework of mathematics and modeling.

CONCLUSION

In 2011, I began serving as the Interim Dean of the College of Agricultural Sciences and Natural Resources at TTU. In 2012, that appointment became permanent, and I served in that role until August 2016, when I become the TTU Interim Provost. The appointment as Provost is now permanent. Is there scientific life once a person enters administration (sometimes referred to as the "dark side")? Yes, I think there is. Since 2012, I have chaired the NASEM Committee on Beef Nutrition, authored or co-authored several journal articles, and spoken at a number of conferences on scientific topics. I will most assuredly have a much lower level of research activity in my current role as Provost, but I still have a few collaborative projects in the works. The more important question related to my work in administration, however, is "has my research background, and specifically the generalist approach I have developed benefitted my work as an administrator? I think the answer is a decided "yes." Having a more general focus has allowed me to get a sense of research and scholarly activities across our campus in a way that I think would have been less effective if I had stayed cloistered in the esoterica of ruminant digestive physiology. But the final thing I would say, particularly to more senior scientists who have a contributed significantly to their disciplinary area and have a mature view of what it takes to be successful in an academic environment, is "Do not rule out administration as a rewarding way to top off your career." We need good university administrators – collegial scholars who will lead and help their fellow faculty members develop a shared vision for the future of their department, college, and university. It is an honorable line of work, and a great fit for scientific generalists.

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